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THE EGGS OF *ANOPHELES PUNCTIPENNIS* AND *ANOPHELES PERPLEXENS* (DIPTERA: CULICIDAE)

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ABSTRACT. The eggs of *Anopheles punctipennis* (Say) and *Anopheles perplexens* Ludlow are morphologically very different, despite close genetic affinity between the species. The egg of *An. punctipennis* is distinct in ventral view by virtue of its wide deck, barely narrowed in the middle of the egg, surrounded by a deep, outwardly flared frill, which conceals the ventral plastron. The deck in *An. perplexens*, in contrast, is much less wide anteriorly and posteriorly and narrows appreciably in the middle of the egg. The frill is low and does not hide the ventral plastron, which appears as two strips flanking the middle deck. Other differences in the lateral profiles and in other structural details are illustrated in electron micrographs and extensive tabulations of morphometric data.

INTRODUCTION

For many years after its original description by Ludlow (1907), the taxonomic identity of *Anopheles* (*Anopheles*) *perplexens* Ludlow continued as a source of controversy. The evolution of opinion on this species was succinctly summarized by Bellamy (1956), who resurrected *An. perplexens* from synonymy with *An. punctipennis* (Say), citing small differences in larval chaetotaxy, wing size and ornamentation, and, particularly, differences in the egg. More recent cytological studies (Kreutzer and Kitzmiller 1971) have demonstrated chromosomal similarities between the two species, but hybridization by forced copulation showed that gene flow between them is impossible (Kreutzer and Kitzmiller 1972).

Although the adults are similar, those who have worked with the two species have consistently reiterated that the eggs are morphologically quite different. These differences have not been illustrated beyond the level of the light microscope, although Aitken (1945) provided a drawing of the egg of *An. punctipennis* and Fritz and Washino (1992) published a photograph showing that eggs of this species may exhibit seasonal changes in morphology. The scale of structure on mosquito eggs is mostly beyond the reach of a stereomicroscope, however, and photographs are subject to limitations of resolution, depth of field, and the presence of undesirable specular reflections in the image. When collections of female *An. punctipennis* and *An. perplexens* were made (by P.E.K.), we fixed and prepared egg batches from several females for scanning electron microscopy. The resulting micrographs are used here to provide descriptions of the eggs of the two species.

MATERIALS AND METHODS

Anopheles punctipennis adult females were collected at human bait on the Santa Fe River, Alachua County, Florida, allowed to blood feed, and later induced to oviposit on wet filter paper. Collections of *An. perplexens* larvae from Lake Panasoffkee, Sumter County, Florida, were reared and the ensuing adults were blood fed and placed in vials for collection of eggs. Sufficient time was allowed for embryonation before the eggs of both species were fixed in alcoholic Bouin's solution. Fixed eggs were dehydrated completely in ethanol and brought to complete dryness by the critical point method. Individual eggs were placed with a fine artist's brush in the required positions on stubs covered with sticky tape and were then sputter-coated with gold/palladium and examined immediately in a Hitachi S-510 scanning electron microscope.

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Table 1. Attributes[1] of eggs of *An. punctipennis* (n = 18) and *An. perplexens* (n = 12), measured from three eggs from each of six or four females, respectively.

Attribute	Mean ± SE[2]	
	An. punctipennis	*An. perplexens*
Linear dimensions[3]		
Egglen	555.9 ± 5.1a	515.0 ± 5.2b
Eggwid	194.2 ± 2.9a	180.2 ± 1.9b
Lenwidrat	2.87 ± 0.04a	2.86 ± 0.02a
Float attributes		
Mnfltlen	327.4 ± 5.1a	322.8 ± 6.3a
Fltpcn	59.1 ± 0.8a	62.8 ± 0.9b
Mnribs	26.3 ± 0.7a	22.9 ± 0.5b
Fltlenprib	12.5 ± 0.2a	14.1 ± 0.2b
Deck dimensions[4]		
Arwhlegg	798.28 ± 16.76a	675.46 ± 10.67b
Arantdk	204.87 ± 3.90a	101.49 ± 2.73b
Arposdk	196.71 ± 3.02a	89.57 ± 3.30b
Artotdk	401.57 ± 5.84a	191.06 ± 5.33b
Antdkpcn	25.8 ± 0.5a	15.0 ± 0.3b
Posdkpcn	24.7 ± 0.4a	13.2 ± 0.3b
Totdkpcn	50.5 ± 0.8a	28.2 ± 0.5b
Dkrat	1.04 ± 0.02a	1.14 ± 0.03b
Ventral plastron[5]		
Arvnplas	—	160.17 ± 4.63
Vnplaspcn	—	23.8 ± 0.8
Noplascel	—	61.9 ± 1.1
Lobed tubercles		
Noantlobtb	6.1 ± 0.2a	5.0 ± 0.2b
Noposlobtb	6.1 ± 0.3a	5.3 ± 0.1a
Totnolobtb	12.2 ± 0.4a	10.3 ± 0.3b
Antposlobrat	1.05 ± 0.04a	0.95 ± 0.04a
Anterior deck tubercles		
Anttbden[6]	70.4 ± 3.1a	72.3 ± 3.4a
Mnanttbar	2.19 ± 0.12a	2.30 ± 0.15a
Mnanttbfm[7]	0.325 ± 0.014a	0.251 ± 0.009b

[1] Abbreviations defined in Appendix.
[2] Means followed by same letter do not differ significantly.
[3] All linear measurements in μm.
[4] All area measurements in $\mu m^2/100$.
[5] Not measured for *An. punctipennis*, see text.
[6] Number in an area of 400 μm^2.
[7] Form factor = 4 × pi × area/perimeter^2.

Morphometric data were collected from micrographs laid on a digitizing tablet used with SigmaScan software (Jandel Scientific, San Rafael, CA). The main series of measurements (Table 1) was made from three eggs from each of six females of *An. punctipennis* (n = 18) and four females of *An. perplexens* (n = 12), using micrographs of the same magnifications and following the same data format and conventions as employed in a previous study (Linley et al. 1993). Certain other attributes (Table 2) were recorded also as before from micrographs of other eggs of the same females. However, the areas occupied by the ventral plastron and the number of component cells were noted only in *An. perplexens* because the ventral plastron in *An. punctipennis* was almost entirely concealed

Table 2. Additional attributes[1] of eggs of *An. punctipennis* and *An. perplexens*. Numbers of measurements (n) derived as indicated in superscripts. All area measurements in μm^2.

Attribute	Mean ± SE[2]	
	An. punctipennis	*An. perplexens*
Dorsal plastron cells[3]	n = 18	n = 12
Celardoplas	280.7 ± 13.2a	362.7 ± 18.9b
Nopordoplas	70.7 ± 3.9a	74.3 ± 7.4a
Porardoplas	1.00 ± 0.07a	1.69 ± 0.21b
Porarpcndoplas	24.2 ± 0.8a	30.8 ± 1.4b
Lobed tubercles[4]	n = 30	n = 20
Antlobtbar	27.9 ± 0.8a	24.3 ± 0.9b
Antnolobes	6.8 ± 0.3a	6.2 ± 0.2a
Micropyle[5]	n = 12	n = 8
Totarmic	660.1 ± 13.5a	481.9 ± 17.7b
Colarmic	383.9 ± 15.5a	294.9 ± 11.2b
Dskarmic	276.1 ± 10.1a	186.9 ± 12.8b
Dskarpcn	41.9 ± 1.6a	38.6 ± 1.5a
Nosect[6]	n = 30	n = 20
	7.5 ± 0.1a	6.9 ± 0.2b

[1] Abbreviations defined in Appendix.
[2] Means followed by same letter do not differ significantly.
[3] Three cells measured from one egg of each female.
[4] Five tubercles measured from one egg of each female.
[5] One micropyle measured from each of two eggs of each female.
[6] Counted from five eggs of each female.

by the well-developed frill (see below). Characters of the individual ventral plastron cells were not measured in either species because they reflected the same information as the dorsal plastron, which was more easily accessible to recording and measurement.

Where means (±SE) are cited in the text, they were derived from an equal number of measurements from eggs of four females in each species. Outer chorionic cell length is the dimension in the longitudinal axis of the egg; width is the circumferential dimension. The descriptive terminology follows Harbach and Knight (1980) except for "plastron cells" (Hinton 1968), "chorionic cell field" (Linley 1989), and "micropylar ray" (Linley et al. 1993). The attribute names (acronyms) used in Tables 1 and 2 are defined in the Appendix.

DESCRIPTIONS

Anopheles punctipennis
(Figs. 1–6)

Size: As in Table 1. *Color*: Black. *Overall appearance*: Boat-shaped in ventral view (Figs. 1A, 2), anterior end slightly wider and more pointed than rounded, slightly tapered at posterior end. Deck wide, extensive, barely to somewhat narrowed in middle, imparting a slipperlike appearance to whole egg (Figs. 1A, 2), frill well developed and erect, especially in middle of egg. Both ventral and dorsal surfaces curved in lateral view (Fig. 1B), float moderately long, midlaterally positioned. Lobed tubercles present at both anterior and posterior ends (Fig. 1A).

Dorsal (lower) and lateral surfaces: Entirely covered with uniformly shaped hexagonal (occasionally pentagonal) plastron-type chorionic cells, with distinct boundaries (Figs. 3B,C; 4). Cell length 20.2–38.6 μm (mean 25.9 ± 0.7 μm, n = 30), width 9.6–15.4 μm (mean 12.7 ± 0.3 μm), area as in Table 2. Structure in cell field consisting of an even array of small, nodular tubercles supported on very short pillars and connected by short bridges with interspaced pores (Figs. 3C, 4), pore characteristics as in Table 2. Chorionic reticulum bounding each cell easily visible, made up of somewhat larger, evenly spaced

Fig. 1. *Anopheles punctipennis*. A, Entire egg, ventral view, anterior end at top; B, entire egg, lateral view, ventral side at left, anterior end at top. Scale = 100 μm.

tubercles topped by a thin central ridge usually connecting to adjacent tubercles (Fig. 3C). At junction with dorsal margin of float, plastron cells with pore area much reduced, float ridges tending to divide some distance from dorsal edge (Fig. 3A). Floats fairly large, about 0.59 of egg length, relatively narrow, other characteristics as in Table 1.

Ventral (upper) surface: Deck wide and continuous, attributes as in Table 1, somewhat to barely perceptibly narrowed in middle (Figs. 1A, 2), both ends of egg similar,

Fig. 2. Single eggs from four females each of *An. punctipennis* and *An. perplexens* (labeled at left), numbered at bottom. Scale = 200 μm.

anterior end not easily distinguished, tending to be only slightly wider. Frill well developed, erect, usually flared outward slightly over middle 0.5 of length (Figs. 1A, 2), composed of pillars, somewhat flattened in longitudinal axis of egg, separated by arched gaps basally, but joined distally (Fig. 5E–G), inner surface with complex branching ridges (Fig. 5H). Strips of ventral plastron present, differing structurally to some degree from dorsal type (Fig. 5E, F), but hidden in ventral view of egg by outwardly recurved frill (Figs. 1A, 5F), area therefore not measured. Entire deck area covered with tubercles, attributes of those on anterior deck measured in Table 1, examples from eggs of four individual females in Fig. 4. Tubercles on remainder of deck very similar to anterior ones, not differing significantly in size or structure (Fig. 5A–C). Each tubercle prominent, shape complex, with convoluted vertical ridges, tops with irregular depressions and cavities, cell floor between tubercles with small ridges and occasional small tubercles (Fig. 5D). Equal number of

lobed tubercles present at both anterior and posterior ends of deck (Table 1), areas and numbers of lobes in anterior group as in Table 2.

Anterior end, micropyle: Anterior end quite rounded, frill not as elevated close to anterior end, deck tubercles somewhat larger in a small area associated with lobed tubercles (Fig. 6A). Lobed tubercles usually more oval than round, elongated in longitudinal axis of egg (Fig. 6A,D), lobes clearly separated, tubercle walls with many small, vertical ridges (Fig. 6F). Characteristics of micropyle as in Table 2, boundaries of plastron cells surrounding it indistinct (Fig. 6B). Micropylar collar close to anterior rim of frill, roughly hexagonal in shape, but outer edge irregular, surface otherwise smooth (Fig. 6B). Inner margin of collar with shallow excavations and radial micropylar rays, forming 7–8 sectors (Fig. 6C).

Posterior end: Somewhat narrower than anterior end, but rounded. Deck wide, frill not as developed as in middle of egg, lobed tubercles surrounded by a small patch of tubercles that are larger than those on remainder of deck (Fig. 6D). Dorsal plastron cells not clearly delineated at very end of egg, meshwork disorganized, often with large gaps (Fig. 6E).

Fig. 3. *Anopheles punctipennis*. A, Dorsal margin of float, middle of egg; B, chorionic (plastron) cells, dosral surface, middle of egg; C, plastron cell detail, dorsal surface. Scale = 50 μm (A,B), = 20 μm (C).

Anopheles perplexens
(Figs. 2, 4, 7–9)

Size: Given in Table 1. *Color*: Black. *Overall appearance*: Boat-shaped in ventral and dorsal view, anterior end somewhat wider and more rounded than posterior end, deck only moderately expanded at both ends, distinctly narrowed in middle of egg between two conspicuous strips of ventral plastron cells (Figs. 2, 7A), lobed tubercles present at both ends. Dorsal surface more curved than ventral in lateral view (Fig. 7B), anterior end markedly deeper than posterior, posterior ventral tip of egg elevated, prominent. Float quite long, positioned toward ventral side, moderately deep (Fig. 7B).

Dorsal (lower) and lateral surfaces: Cells of dorsal plastron regularly pentagonal or hex-

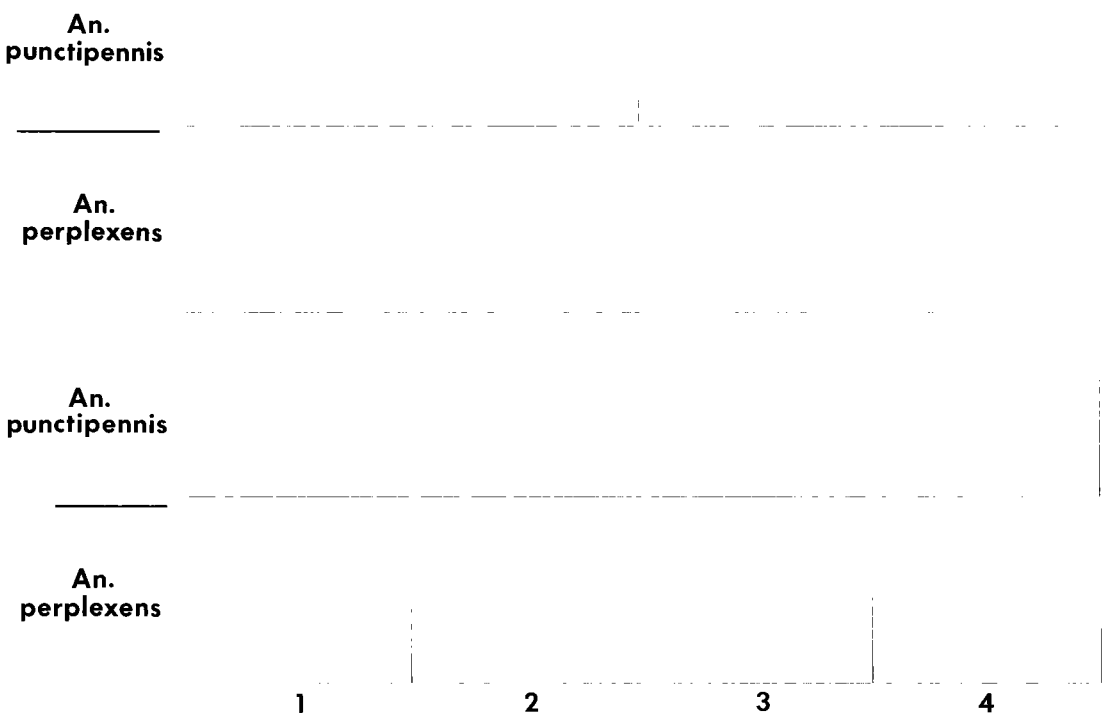

Fig. 4. Tubercles of the anterior deck (upper) and plastron cells (lower) of *An. punctipennis* and *An. perplexens* (labeled at left); one egg from each of four females (numbered at bottom). Scale = 20 μm (plastron, lower), = 10 μm (tubercles, upper).

agonal, edges straight, chorionic reticulum of each cell pronounced (Fig. 8G). Cell length 24.1–34.2 μm (mean 26.6 ± 0.6 μm, n = 20), width 12.3–20.3 μm (mean 15.8 ± 0.6 μm), area in Table 2. Interior plastron surface of each cell with nodular tubercles and interspersed pores (pore data in Table 2), some coalesced, occasionally crossing cell boundaries, which are composed of larger tubercles joined by a thin, raised central ridge (Figs. 4, 8H). Network in cells adjacent to dorsal float margin more closely knit, pores fewer and smaller, float ridges dividing some distance from margin (Fig. 8F). Float quite long, about 0.63 of length of egg, attributes as in Table 1.

Ventral (upper) surface: Deck (quantitative attributes as in Table 1) continuous along length of egg, but quite narrow overall, somewhat wider anteriorly and posteriorly, variably narrowed in middle between two strips of ventral plastron (Figs. 2, 7A). Frill deeper in anterior and posterior 0.25 of deck, low in middle of egg, along inner sides of plastron strips (Fig. 7A). Strips (attributes in Table 1) separated from inner sides of floats, their constituent chorionic cells rendered easily visible by unusually high, thin reticulum, cell surfaces less open than in dorsal plastron, pores fewer (Fig. 8E). Entire deck covered with tubercles, very similar in form overall (Fig. 8A–C), measurements for anterior ones as in Table 1, examples from four females in Fig. 4. Middle deck tubercles significantly smaller in area than anterior ones and smaller, though not significantly so, than those at posterior end. Shape of tubercles complex, sides consisting of prominent, more or less radially spreading ridges, tops with deep indentations (Fig. 8A–D), cell floor between tubercles also ridged, with occasional small tubercles (Fig. 8D). Both ends of deck with lobed tubercles

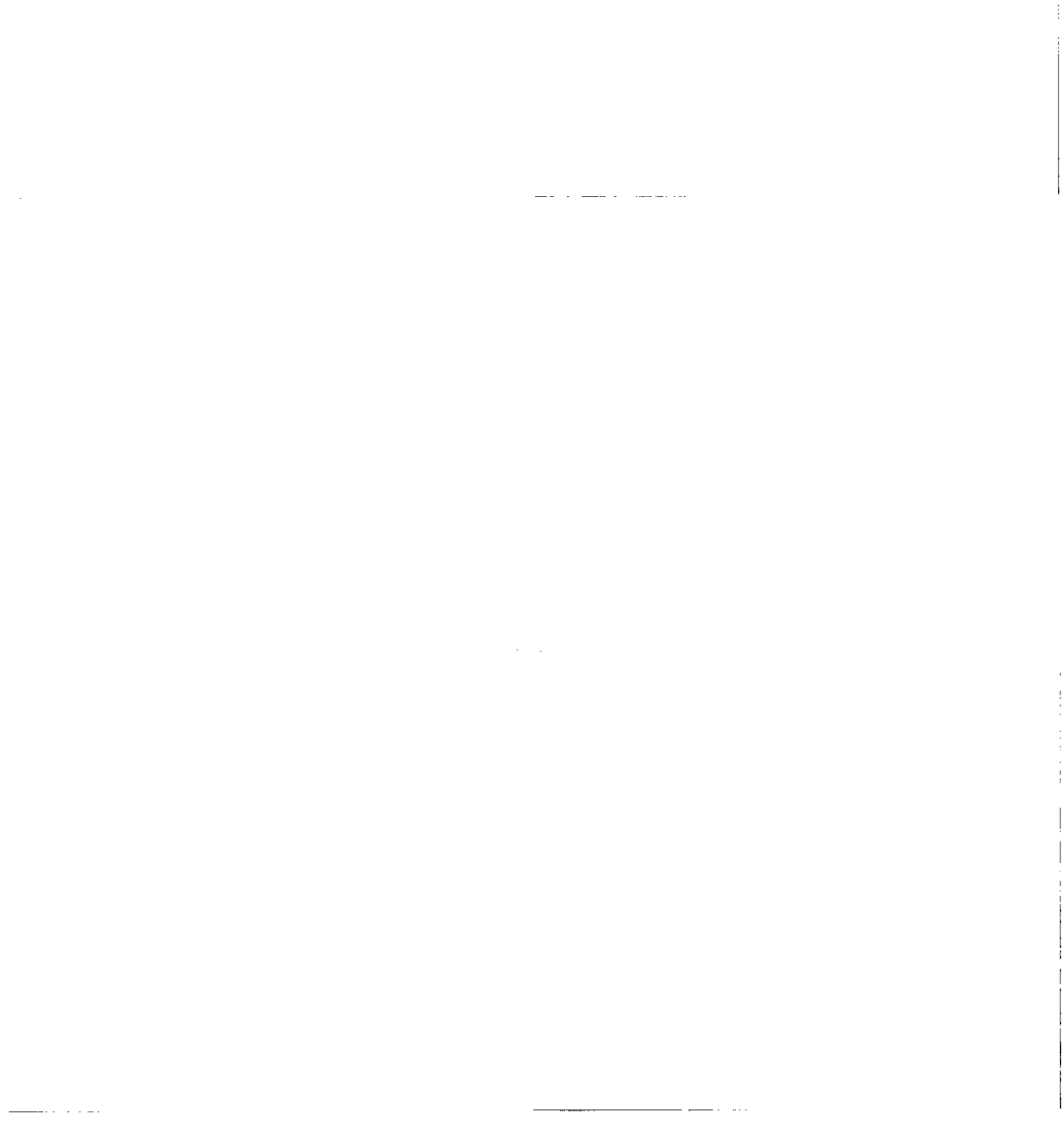

Fig. 6. *Anopheles punctipennis.* A, Extreme anterior end, ventral surface; B, anterior end, end-on view, with micropylar apparatus; C, detail of micropylar apparatus; D, extreme posterior end, ventral surface; E, posterior end, end-on view; F, detail of lobed tubercles, anterior end of ventral surface. Scale = 50 μm (A,B,D,E), = 10 μm (C,F).

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Fig. 5. *Anopheles punctipennis.* A, Tubercles, anterior deck; B, tubercles, middle deck; C, tubercles, posterior deck; D, detail of tubercle structure, anterior deck; E, junction of ventral plastron and lateral plastron at anterior end of float; F, detail of ventral plastron, middle of egg, float at bottom, frill and middle deck at top; G, spatial relationships of middle deck (bottom), frill and float (top); H, detail of inside wall of frill, middle of egg. Scale = 20 μm (E–G), = 10 μm (A–C,H), = 5 μm (D).

Fig. 7. *Anopheles perplexens.* A, Entire egg, ventral view, anterior end at top; B, entire egg, lateral view, ventral side at left, anterior end at top. Scale = 100 μm.

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Fig. 8. *Anopheles perplexens.* A, Tubercles, anterior deck; B, tubercles, middle deck; C, tubercles, posterior deck; D, detail of tubercle structure, anterior deck; E, chorionic (plastron) cell detail, middle of ventral surface; F, dorsal margin of float, middle of egg; G, chorionic (plastron) cells, dorsal surface; H, plastron cell detail, dorsal surface. Scale = 50 μm (G), = 20 μm (E,F,H), = 10 μm (A–C), = 5 μm (D).

Fig. 9. *Anopheles perplexens.* A, Extreme anterior end, ventral surface; B, anterior end, end-on view, with micropylar apparatus; C, detail of micropylar apparatus; D, extreme posterior end, ventral surface; E, posterior end, end-on view; F, detail of lobed tubercles, anterior end of ventral surface. Scale = 20 μm (A,B,D,E), = 10 μm (C,F).

(data in Table 1), about equal numbers at each end, size and numbers of lobes in anterior group given in Table 2, lobes in each tubercle usually clearly separated (Fig. 9F), tubercle walls roughened by small, close ridges.

Anterior end, micropyle: Deck tubercles around anterior lobed tubercles only slightly larger than on remainder of deck, which is rather narrow (Fig. 9A). Plastron flanking deck on either side with unusually deep reticulum, clearly delineating cells (Fig. 9A). Micropylar collar irregular in outline, smooth-surfaced, slightly separated from prowlike anterior margin of frill (Fig. 9B). Deep reticulation of plastron cells close to anterior end clearly apparent, extending almost to micropylar collar (Fig. 9B). Interior margin of collar with regular, shallow indentations associated with long micropylar rays dividing disk into 6–8 sectors (complete micropyle data in Table 2), disk surface slightly rough, orifice seated within a small mound (Fig. 9C).

Posterior end: Narrower than anterior end, rather pointed (Fig. 2), fairly narrow deck again allowing prominently reticulated plastron cells on each side to be seen easily, deck tubercles immediately surrounding lobed tubercles only slightly larger than on general surface of deck (Fig. 9D). End-on view shows posterior end of egg rather sharp, raised, lobed tubercles borne on a slight mound (Figs. 7B, 9E), boundaries of plastron cells becoming invisible at very end of egg (Fig. 9E).

DISCUSSION

These eggs have been described with rather extensive tabulations of characters, not only for the sake of completeness, but with a view possibly to incorporating them in a multivariate analysis with other species that they resemble. It may prove useful to do this particularly in the case of *An. perplexens,* as its eggs are very similar to those of at least one other species, *An. crucians* Wiedemann, whereas the *An. punctipennis* egg is distinctive in a number of respects, as will be discussed shortly. The close similarity between eggs of *An. perplexens* and *An. crucians* has become apparent from a recent examination (Linley and Kaiser, unpublished) of the eggs of four species in the Crucians Complex newly recognized by electrophoretic methods (Kaiser, unpublished). In overall structure and appearance, the egg of *An. perplexens* is difficult to distinguish from the *An. crucians* material, the main visible difference being the easily visible ventral plastron reticulation in *An. perplexens.* A full comparison of these eggs will be presented at a later date.

The distinction between *An. perplexens* and *An. punctipennis* is easily made, as noted previously in the literature (Bellamy 1956, Kreutzer and Kitzmiller 1972). In ventral aspect, the wide deck, barely narrowed in the middle of the egg and with deep erect frill, as well as the absence of visible ventral plastron, immediately distinguishes the slipper-like egg of *An. punctipennis.* It should be noted, however, that "winter" eggs of *An. punctipennis,* from the photograph shown by Fritz and Washino (1992), have an extensive plastron covering much of the ventral surface and would be rather different in appearance. Other differences between *An. perplexens* and *An. punctipennis* are seen in lateral profile, where the respective frill development is again obvious and where the relatively greater anterior depth of *An. perplexens* is apparent, along with its more elevated posterior tip.

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APPENDIX

Definitions of abbreviations (acronyms) of attributes listed in Tables 1 and 2.

Antdkpcn—area of anterior deck as % area whole egg
Antlobtbar—mean anterior lobed tubercle area
Antnolobes—anterior lobed tubercle mean number of lobes
Antposlobrat—anterior/posterior lobed tubercles ratio
Anttbden—anterior deck tubercle density
Arantdk—area of anterior deck
Arposdk—area of posterior deck
Artotdk—area of total deck (anterior + posterior)
Arvnplas—area of the ventral plastron
Arwhlegg—area of whole egg (ventral view)
Celardoplas—mean chorionic cell area, dorsal plastron
Colarmic—collar area of micropyle
Dkrat—ratio of anterior deck area/posterior deck area
Dskarmic—disk area of micropyle
Dskarpcn—disk area as % total micropylar apparatus area
Egglen—egg length
Eggwid—egg width (widest point, across floats)
Fltlenprib—mean float length/mean number of ribs
Fltpcn—mean float length as % of egg length
Lenwidrat—egg length/width ratio
Mnanttbar—mean anterior deck tubercle area
Mnanttbfm—mean anterior deck tubercle form factor
Mnfltlen—mean float length (of the 2 floats)
Mnribs—mean number of ribs (of the 2 floats)
Noantlobtb—number of anterior lobed tubercles
Noplascel—number of ventral plastron chorionic cells
Nopordoplas—mean number of cell pores, dorsal plastron
Noposlobtb—number of posterior lobed tubercles
Nosect—number of sectors in micropylar disk
Porardoplas—mean individual pore area, dorsal plastron
Porarpcndoplas—total pore area as % cell area, dorsal plastron
Posdkpcn—area of posterior deck as % area whole egg
Totarmic—total area of micropylar apparatus
Totdkpcn—area of total deck as % area whole egg
Totnolobtb—total number of lobed tubercles
Vnplaspcn—area of ventral plastron as % area whole egg