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BLOOD BIOCHEMISTRY AND RELATIONS AMONG *PODOCNEMIS* TURTLES (PLEURODIRA, PELOMEDUSIDAE)

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Abstract—Agglutination, precipitation and electrophoresis of hemoglobin and serum point to a core of five *Podocnemis* species (*P. erythrocephala, P. expansa, P. lewyana, P. unifilis* and *P. vogli*), with a sixth species (*P. sextuberculata*) fringing this cluster.

2. In addition, the data indicate that *P. dumeriliana* and *P. madagascariensis* not only are generically distinct from the other six species, but also are not closely related to each other. The name *Peltocephalus* Dumeril & Bibron, 1835, is available for *Podocnemis dumeriliana*, while *Erymnochelys* Baur, 1888, should be used for *P. madagascariensis*.

INTRODUCTION

The pelomedusid turtle genus *Podocnemis* usually is thought to consist of eight living species, seven of them from northern South America and one from Madagascar (Williams, 1954a; Wermuth & Mertens, 1961; Pritchard, 1967; Mittermeier & Wilson, 1974). However, the status of two of these species has never been clear. Williams (1954b, c) and Smith & James (1958) placed the Madagascan species in its own genus, *Erymnochelys* Baur, 1888; and Tronc & Vuillemin (1974) recently resurrected the name once again. In addition, the South American species usually referred to as *Podocnemis dumeriliana* sometimes has been placed in the monotypic genus *Peltocephalus* (Williams, 1954b, c; Fretey, 1975, 1977).

Pelomedusid turtles have received only scant biochemical (serological) treatment (Frair, 1964). In this present paper, we attempt to clarify relationships among the *Podocnemis* by providing data on their blood proteins and those of other pelomedusids and selected chelid turtles.

MATERIALS AND METHODS

Blood was obtained by aseptic cardiocentesis, the needle being inserted anterior to a hind leg. In two *Podocnemis expansa* cervical venipuncture was performed. Serum was stored at −30°C; hemolysate reagent and most electrophoresis supplies were from Helena Laboratories Corporation. Tris–EDTA–boric acid buffer at pH 8.4 (EDTA—ethylenediaminetetracetic acid) and ionic strength 0.025 was used for hemoglobin, and merthiolated Tris–barbital buffer at pH 8.8 and ionic strength 0.05 used for serum. All electrophoresis was run in cellulose acetate plate at constant 180 V for 15 min.

Two rabbit antisera for each of *Podocnemis dumeriliana, P. madagascariensis* and *P. unifilis* were produced against whole serum using Freund's complete adjuvant. Passive hemagglutination with a tanned rabbit cell technic was performed according to Herbert (1973); agar double immunodiffusion plates were from Cappel Laboratories, Inc. Protein concentrations were determined with biuret reagent and solutions appropriately diluted to equivalent proportions for each test. The above methods were selected for specificity and sensitivity using mixed antigens and small volumes of reactants.

The numbers of specimens utilized were *P. dumeriliana*, 2; *P. erythrocephala*, 4; *P. expansa*, 5; *P. lewyana*, 2; *P. sextuberculata*, 2; *P. unifilis*, 7; *P. vogli*, 3; all others, 1. Serum was thawed and used within 21 months from the time it originally was frozen. An exception was *Chelus fimbriatus* stored at −20°C for as long as $7\frac{1}{2}$ yr. Most of the turtles used are still being maintained alive.

RESULTS

Electrophoresis

Electrophoretic patterns of hemoglobin from fresh washed cells of *Podocnemis* species were similar and had 2–7 lines regardless of whether hemolysis was performed utilizing distilled water or hemolysate reagent. Two widely separated dark bands of *P. erythrocephala* were positioned like the two main bands of *P. unifilis* (which also had two or three lighter bands). *P. vogli* and *P. expansa* had slightly more widely separated dark bands and one or two lighter ones. *P. lewyana* differed from the above four in that the heavy anodal component was more slowly moving, a feature likewise exhibited by *P. sextuberculata. P. sextuberculata* also resembled *P. madagascariensis* in having a cathodal duplex. The *P. dumeriliana* pattern differed from all the others and had seven narrow lines, the leading heavy anodal one traveling faster than bands for any other *Podocnemis*.

Serum electrophoregrams are pictured in Fig. 1. In addition to recent specimens shown here, electrophoretic patterns for some of the same species

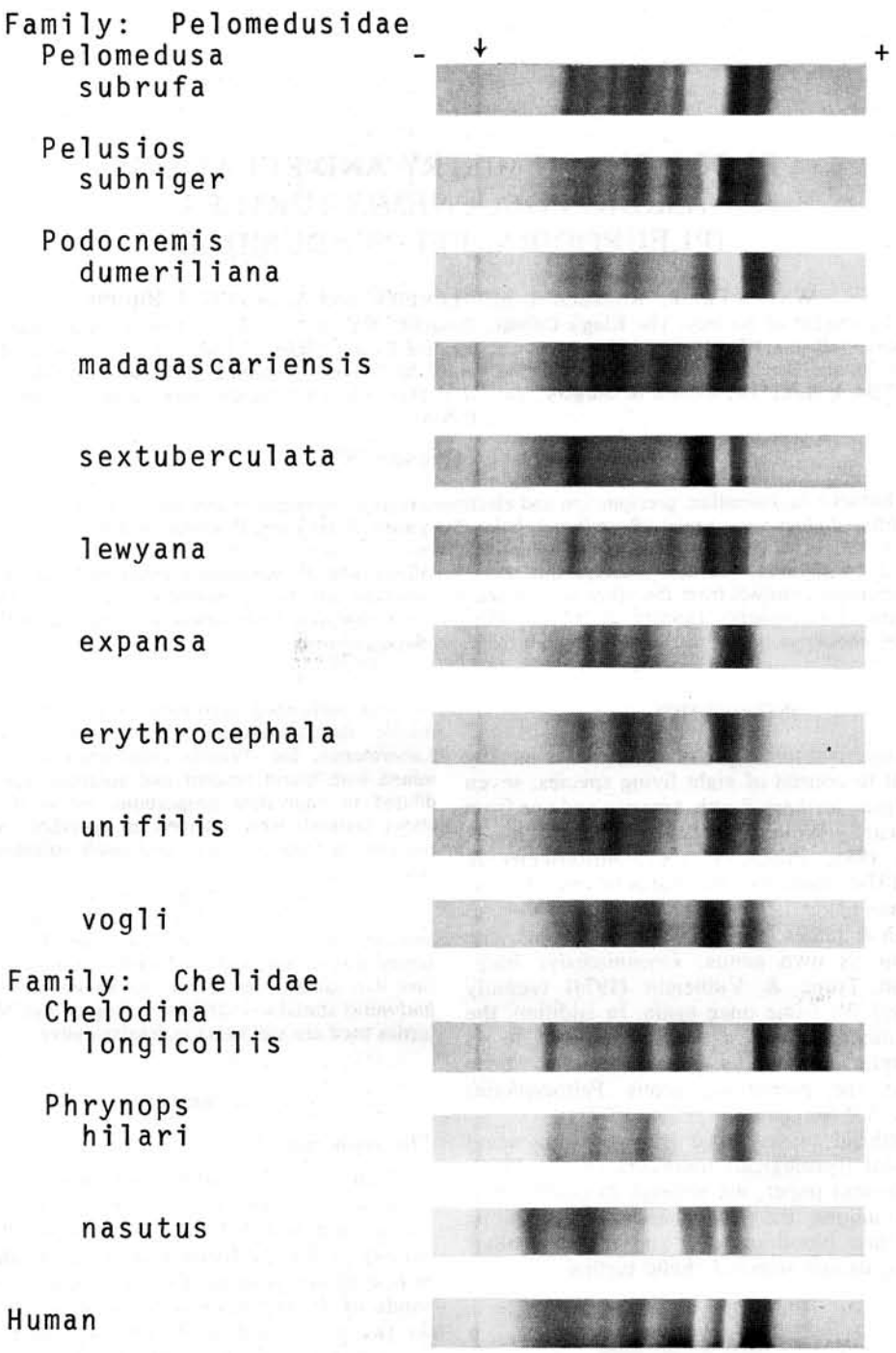

Fig. 1. Photographs of cellulose acetate plate electrophoregrams. ↓ where serum was applied.

were obtained using serum which had been frozen at −20°C for as long as 15 yr, with the result that lines were similarly positioned but more diffuse. The resemblance of *P. erythrocephala*, *P. unifilis* and *P. vogli* patterns is apparent. *P expansa* is similar to these but has a single rather than a duplex anodal component. *P. lewyana* and *P. sextuberculata* have a similar trailing cathodal line. A trailing but more diffuse cathodal line is seen in the *P. madagascariensis* pattern. *P. dumeriliana* has the most rapidly moving anodal albumin and longest electrophoretic pattern among the *Podocnemis*.

Agglutination

Table 1 shows a summarized condensation of results with the reference antigen values underlined. *P. expansa* and *P. vogli* consistently were high and the amount they exceed 100% of the reference antigen is within the margin of error of about ±5%. In reciprocal tests involving anti-Pd, anti-Pm, and anti-Pu, the *P. dumeriliana* is most

Table 1. Agglutinations of rabbit erythrocytes coated with turtle serum proteins

Antigens on erythrocytes	Titers with antibodies against: Pd*	Pm	Pu
Podocnemis			
dumeriliana	100	68	74
erythrocephala	97	84	95
expansa	102	94	100
lewyana	100	83	100
madagascariensis	84	100	84
sextuberculata	90	66	92
unifilis	86	90	100
vogli	99	89	103
Pelomedusa subrufa	80	78	77
Pelusios subniger	98	78	76
Chelus fimbriatus	91	76	78
Hydromedusa tectifera	74	74	76
Phrynops			
gibbus	83	78	78
hilari	66	70	78
nasutus	83	76	78
Chelodina longicollis	82	78	74
Elseya latisternum	66	70	64
Chelydra s. serpentina	78	70	70

* Pd—*Podocnemis dumeriliana*; Pm—*P. madagascariensis*; Pu—*P. unifilis*. Titers are percentages of reference antigen values and are to be compared only in the vertical columns. The pooled standard deviation of all values is 5.45. Except for *P. madagascariensis* all *Podocnemis* are from South America. *Pelomedusa* and *Pelusios* are from the Africa–Madagascar region. *Chelus*, *Hydromedusa* and *Phrynops* are South American. *Chelodina* and *Elseya* inhabit Australia and New Guinea, and *Chelydra* is a North American cryptodire.

distant from *P. unifilis* and *P. madagascariensis* (*P. sextuberculata* also reacts weakly with anti-Pm). Anti-Pu places *P. madagascariensis* between *P. dumeriliana* and the other *Podocnemis*, distinguishing *P. dumeriliana* from all other forms tested and shows that this animal is no closer to typical *Podocnemis* than are many other pleurodiran genera.

Anti-Pm indicates that *P. madagascariensis* is closest to *P. expansa*, *P. unifilis* and *P. vogli*; but *P. erythrocephala* and *P. lewyana* are not much closer to it than are *Pelusios*, *Pelomedusa* and some chelids. Also, many chelids appear more like *Podocnemis madagascariensis* than does *P. sextuberculata*.

With anti-Pd, although certain *Podocnemis* values are high, some non-*Podocnemis* titers also are elevated. Even if some artifact were introduced in obtaining *Pelusios* and *Chelus* titers,* there are other non-*Podocnemis* values in the 80s close to the lower *Podocnemis* titers. Therefore, *P. dumeriliana* clearly is not associated with other *Podocnemis*.

* Recent agglutination tests with two anti-*Pelusios* antisera gave high titers with *Pelomedusa*, medium with many chelids and *Podocnemis* except *P. dumeriliana*, and low with *P. dumeriliana* (and *Elseya*). Because of the low value for *P. dumeriliana*, we consider the Table 1 anti-Pd *Pelusios* value of 98 as tentative.

Double diffusion

Thirty double diffusion plates, each with five or six peripheral wells, were set up using the same antisera and most of the same sera employed in agglutination tests. Sera of *Chelus*, *Hydromedusa*, *Phrynops gibbus*, *Elseya* and *Chelydra* were not utilized. A sample plate is pictured in Fig. 2. Visual evaluations (sometimes with low magnification) were based upon the number and width of precipitation lines in agar. Observations were made during a span of about 1 week, and results generally were concordant with findings in the more quantitative agglutination assays. Usually suggested refinements of the order of values in Table 1 were within the margins of error for the agglutination procedure. An exception was the anti-Pd with *Phrynops hilari* which had a precipitation pattern like (or possibly slightly heavier than) *P. nasutus*, thus indicating that the *P. hilari* agglutination titer of 66 is too low. In the various plates, patterns of identity commonly were seen between sera from similar organisms. Spurring appeared between the more diverse species, for example, between *Chelodina* or *Podocnemis dumeriliana* and various *Podocnemis*. In reactions with all six of the antisera, *Pelomedusa* and *Pelusios* reacted more strongly than the chelids *Phrynops hilari* and *P. nasutus*. Among *Podocnemis* the greatest difference between the two titers averaged to obtain each mean given in Table 1 was for *P. sextuberculata* with anti-Pd. One anti-Pd serum reacted strongly with *P. sextuberculata* and the other more weakly in both agglutination (100 and 79) and double diffusion tests.

Among *Podocnemis*, the species reacting weakest with anti-Pm were as given in Table 1, except that *P. dumeriliana* tended to show somewhat less precipitate (and more spurring) than *P. sextuberculata*; nevertheless, both of these showed slightly heavier reactions than did the chelids *Phrynops hilari*, *P. nasutus* and *Chelodina*. However, as Table 1 shows, *Podocnemis madagascariensis* consistently was closer to *Pelusios* and *Pelomedusa* than to *Podocnemis sextuberculata* and *P. dumeriliana*. Also, when compared to *P. madagascariensis*, *P. erythrocephala* exhibits quantitative precipitation reactions like the group composed of *P. vogli*, *P. unifilis* and *P. expansa*.

The anti-Pu in agglutination (Table 1) did not distinguish between *P. dumeriliana* and *Chelodina*, but the precipitation pattern appeared slightly heavier for *P. dumeriliana*. Also with anti-Pu the *P. unifilis*, *P. vogli*, *P. expansa* and *P. lewyana* patterns looked more alike than the increasingly dissimilar ones of *P. erythrocephala*, *P. sextuberculata*, *P. madagascariensis* and *P. dumeriliana*. Importantly, the precipitation confirmed the tentative conclusion from agglutination that *Pelomedusa* and *Pelusios* are slightly closer to *Podocnemis unifilis* than are *Chelodina* and *P. dumeriliana*. With anti-Pd, although *P. erythrocephala* had a heavy precipitate, the pattern was slightly different than for *P. lewyana*, *P. unifilis*, *P. expansa* and *P. vogli*.

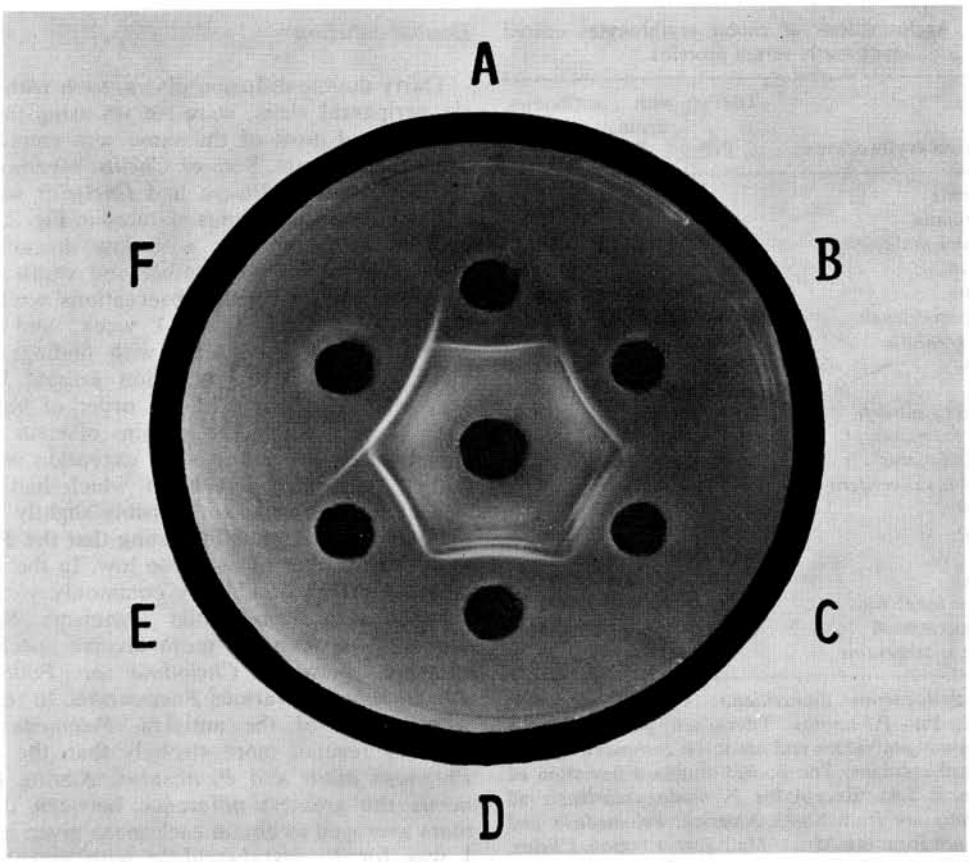

Fig. 2. Photograph of double diffusion plate showing precipitation lines in agar. Center well, anti-Pu; A, *Podocnemis dumeriliana*; B, *P. madagascariensis*; C, *P. sextuberculata*; D, *P. lewyana*; E, *P. expansa*; F, *P. unifilis*. Especially note lines of identity between C and D, D and E; likewise the spurs between A and B, B and C. The large spur is lighter between E and F than between F and A.

DISCUSSION

Our conclusions from the hemoglobin results are somewhat more tentative than others because they are based upon only a single protein; and the methodology, though consistently performed across species, did not eliminate some possible polymerization. However, both hemoglobin and serum electrophoretic patterns as well as agglutination and precipitation all point to the existence of a core of similar species consisting of *Podocnemis expansa*, *P. vogli*, *P. unifilis*, *P. erythrocephala* and *P. lewyana* (this list from *P. expansa* generally reflects decreasing endowment of features in common). *P. sextuberculata* tends to fringe the cluster but is closer than *P. madagascariensis*.

P. dumeriliana has a unique hemoglobin polymorphism and the fastest wide anodal serum electrophoretic line. It evidences as little or less similarity to the core (as represented by *P. unifilis*) than do *Pelomedusa*, *Pelusios* and chelids. Although high values in reactions with *Podocnemis dumeriliana* antisera indicate proximity of *P. dumeriliana* to members of the core, there also are high values with *Pelusios* and *Chelus*.

We feel that these data clearly indicate that *Podocnemis dumeriliana* is a distinct genus, a finding that is also supported by our morphological and chromosomal studies (Mittermeier *et al.*, in preparation; Rhodin *et al.*, in press) and is in agreement with Williams (1945b, c) and Fretey (1975, 1977). We therefore resurrect the name *Peltocephalus* Dumeril & Bibron, 1835, for this animal. The agglutination results also provide support for resurrection of the genus *Erymnochelys* Baur, 1888, for *Podocnemis madagascariensis*. However, this animal clearly has more in common with the *Podocnemis* than does *Peltocephalus*. Consequently, we do not agree with the proposal by Smith & James (1958) that *Erymnochelys* be placed in its own subfamily.

Several authors have suggested that there is a close relationship between *Erymnochelys* and *Peltocephalus* (Baur, 1890; Siebenrock, 1902; Müller, 1935; Williams, 1954c), but our blood studies do not indicate this to be true. Reciprocal reactions between *Peltocephalus* and *Erymnochelys* with their antisera place these two species no closer to each other than either is to some chelids, *Pelusios* and probably *Pelomedusa*. Nonetheless, the data do show that *Erymnochelys*, *Peltocephalus* and the six *Podocnemis* have enough in common to be placed in the same family and probably the same subfamily. When morphological and chromosomal data are also taken into account, the subfamilial category appears to be most appropriate. We therefore suggest that these three

genera be placed together in the subfamily Podocneminae, a name first proposed by Smith & James (1958) for the seven South American species and later expanded by Wood (1971) to include *Erymnochelys*.

On morphological grounds, Mittermeier & Wilson (1974) suggested that *Podocnemis erythrocephala* is most closely related to *P. unifilis*. Electrophoretic patterns for hemoglobin and serum support this contention, but antigen–antibody tests indicate that several other species are also very close to *P. unifilis*.

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