VARIATION IN NUMBERS OF SCLERAL OSSICLES AND THEIR PHYLOGENETIC TRANSFORMATIONS WITHIN THE PELECANIFORMES

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ABSTRACT.—We examined scleral rings from 44 species of Pelecaniformes and found nonrandom variation in numbers of scleral ossicles among genera, but little or no variability within genera. *Phaethon, Fregata,* and *Pelecanus* retain the primitive 15 ossicles per ring, while the most recent common ancestor of the Sulae (Phalacrocoracidae, *Anhinga,* and Sulidae) is inferred to have had a derived reduction to 12 or 13 ossicles. Within the Sulidae, *Sula* (*sensu stricto*) exhibits further reduction to 10 ossicles. These patterns of ossicle reduction are congruent with both Cracraft's hypothesis of pelecaniform relationships (1985) and that of Sibley et al. (1988). The presence of scleral rings in museum specimens is significantly greater for *Phaethon* and *Fregata,* and less for *Pelecanus,* than would be expected from a random distribution. We conclude that the scleral ring is of potential systematic importance, and we make recommendations for its preservation in museum collections. *Received 11 August 1988, accepted 25 January 1989.*

THE scleral ring (*annulus ossicularis sclerae*) is a ring of small overlapping platelike bones, the scleral ossicles (*ossicula sclerae*), found within the sclera in the corneal hemisphere of the eye between the retinal margin and the conjunctival ring (Edinger 1929, Martin 1985). The function of the scleral ring as a whole, and the individual ossicles in particular, is poorly known. Two functions are most often proposed for the scleral ring: first, as support and protection of the eye in the region where it is found; alternatively, as an attachment for the ciliary muscles, specifically, the anterior corneal muscle (*m. cornealis anterior*), which suggests a role in corneal accommodation (Lemmrich 1931, King and McLelland 1984, Martin 1985). Curtis and Miller (1938) also suggested that eccentricity of the ring aperture may facilitate binocular vision. The arrangement of the ossicles themselves is even more problematic, although Lemmrich (1931) proposed that having a scleral ring composed of overlapping plates permits growth of the ring during ontogeny. Lemmrich also proposed that the differences in ossicle number among taxa may be attributed to "growth organization" (*Organisationmerkmal*) particular to taxa. Because the scleral ring exists in a wide variety of vertebrates (Edinger 1929) including nonavian dinosaurs (references in de Queiroz and Good 1988), its presence in birds appears to be a retained primitive character. Therefore, any explanation of the ring's function within birds must also consider its widespread occurrence in Vertebrata. Whatever function the scleral ring may serve, the number of ossicles per ring varies in a nonrandom pattern among some avian taxa (Lemmrich 1931, Curtis and Miller 1938, de Queiroz and Good 1988).

The use of the scleral ring as a systematic character in birds has been limited. Studies have been descriptive surveys, conducted primarily at the ordinal or familial levels, on ring morphology, function, and variation in ossicle number and position (e.g. Lemmrich 1931, Curtis and Miller 1938). While conducting two independent research projects, one on the scleral ossicles and phylogenetic relationships of the Hoatzin (de Queiroz and Good 1988), and the other on the phylogenetic relationships within the Sulidae (Olson and Warheit 1988, Warheit in prep.), we discovered what appears to be phylogenetically informative patterns of variation in numbers of scleral ossicles among the pelecaniform genera.

We summarized the numbers of scleral ossicles for extant species of Pelecaniformes, and examined these patterns in the context of current hypotheses about pelecaniform relationships. As a result of our survey, we also found that scleral rings are not satisfactorily main-

tained in osteological collections, and are absent even in some specimens that are considered "complete." Accordingly, we make recommendations for the preparation of scleral rings in osteological specimens.

MATERIALS AND METHODS

We sampled 695 skeletons from 51 species of pelecaniforms. The number of specimens sampled for a particular species was determined by its availability in the collections. For species with a limited number of skeletons, all specimens were examined. If the sample size for a species was large ($n > 20$), skeletons were chosen randomly, but with an effort to maintain equal numbers of males and females.

We recorded the number of complete rings present (0, 1, or 2) for each specimen. When ossicle overlap is extreme, some plates may be visible on only one side of the ring. Therefore, the number of ossicles that constitute a ring was determined by counting plates on both the inner and outer sides. For specimens with two rings, both rings were examined. In most cases (87%) the number of ossicles in the left and right rings was the same, but occasionally the number differed (see Curtis and Miller 1938 for univariate statistics and discussion of constancy of scleral ossicle numbers between right and left eyes for various taxa of birds).

Mean counts were recorded for those specimens with both left and right rings preserved. For example, if the number of ossicles in the left ring was 14 and the right ring was 15, a count of 14.5 was used for that specimen. This was done because using the total number of scleral rings to calculate the mode, rather than the preferred total number of specimens, would not only violate the assumption of independent samples, but would also bias the mode towards specimens with two rings over those with only one ring.

We summarized the data in two ways. First, modal numbers of ossicles are reported for 44 of the 51 species surveyed (no scleral rings were available for 7 species). Second, the availability of ossicles for each species was determined by comparing the number of specimens containing ossicles (either one or two) to the total number of specimens examined. Specimens with broken or disarticulated rings were excluded.

RESULTS

Variation in the number of scleral ossicles.—The modal numbers of ossicles per scleral ring for 44 species of pelecaniforms are listed in Table 1. Ossicle numbers are distributed in a phylogenetically informative pattern within and among pelecaniform genera. Within genera there is little variability in ossicle number. Within *Phalacrocorax* (*sensu lato*; see below), however, we found 14 species with a modal number of 13 ossicles, 4 species with 12 ossicles, and 3 species with 12 or 13 ossicles (the modal number of ossicles could not be resolved for the latter 3 species because of small sample sizes). Similarly, modal numbers of ossicles vary among the species within the single sulid genus (*Sula*) recognized most recently by Nelson (1978) and the American Ornithologists' Union (A.O.U. 1983), but does not vary within each of the three sulid genera recognized by Olson and Warheit (1988).

Availability of scleral rings.—Availability of scleral rings was not distributed randomly among the genera ($\chi^2 = 42.8$, $P < 0.001$; Table 1); however, an analysis of the residuals of the Chi-square (see Haberman 1973) shows that not all genera deviate significantly from independence. The number of scleral rings present for both *Phaethon* and *Fregata* are significantly greater ($\chi^2 = 14.83$, SR (standarized residual adjusted by estimate of variance) = 1.76, $P < 0.05$; $\chi^2 = 27.54$, SR = 2.13, $P < 0.05$; respectively), while the number of rings present for *Pelecanus* is significantly less ($\chi^2 = 37.28$, SR = −1.78, $P < 0.05$) than expected from a random distribution. The number of scleral rings present in the other genera was not significantly different from a random distribution ($P > 0.05$). The probabilities associated with the number of rings absent were not significant ($P > 0.05$) for all genera, but *Phaethon*, *Fregata*, and *Pelecanus* approached significance ($P = 0.10$), with *Phaethon* and *Fregata* having fewer rings absent, and *Pelecanus* having more rings absent than expected.

DISCUSSION

Based on comparisons with ratites, tinamous, and nonavian dinosaurs (de Queiroz and Good 1988), the primitive number of scleral ossicles for neognath birds appears to be 14 or 15. Numbers in this range are taxonomically widespread within Neognathae (Lemmrich 1931, Curtis and Miller 1938, de Queiroz and Good 1988). Cracraft (1985) argued that the pelecaniforms are monophyletic and suggested that the Procellariiformes, Sphenisciformes, and Gaviiformes are the most significant outgroups. These outgroup taxa possess either 14 or 15 scleral ossicles (Lemmrich 1931, Curtis and Miller 1938). Sibley et al. (1988) suggested that the Falconiformes and Charadriiformes are the closest outgroups

TABLE 1. Total number of specimens examined, number (and percentage) of specimens with ossicles, and modal number of ossicles for species of Pelecaniformes. Data associated with each genus heading equals the totals for all the species within that genus.

Taxon	Specimens		Modal no.
	Total no.	No. with ossicles (%)	
PHAETHONTIDAE			
Phaethon	35	24 (69)	15
P. aethereus	4	2 (50)	14–15
P. lepturus	8	5 (63)	15
P. rubricauda	23	17 (74)	15
FREGATIDAE			
Fregata	65	43 (66)	15
F. aquila	11	5 (45)	15
F. ariel	24	22 (92)	14–15
F. magnificens	8	1 (13)	14
F. minor	22	15 (68)	15
PELECANIDAE			
Pelecanus	88	22 (25)	15
P. conspicillatus	4	0 (0)	—
P. crispus	2	0 (0)	—
P. erythrorhynchos	19	7 (37)	15
P. occidentalis	46	12 (26)	15
P. onocrotalus	8	2 (25)	16
P. philippensis	2	1 (50)	14
P. rufescens	7	0 (0)	—
PHALACROCORACIDAE			
Phalacrocorax	274	123 (45)	13
P. africanus	7	2 (29)	13
P. aristotelis	1	0 (0)	—
P. atriceps	24	12 (50)	13
P. auritus	34	20 (59)	13
P. bougainvillii	14	8 (57)	12
P. capensis	14	14 (100)	13
P. carbo	23	8 (35)	13
P. carunculatus	2	0 (0)	—
P. coronatus	9	9 (100)	13
P. gaimardi	2	1 (50)	12
P. harrisi	8	2 (25)	13
P. magellanicus	9	5 (56)	12
P. melanoleucos	13	2 (15)	12–13
P. neglectus	8	8 (100)	13
P. olivaceus	24	15 (63)	13
P. pelagicus	20	2 (10)	12–13
P. penicillatus	21	4 (19)	13
P. punctatus	7	0 (0)	—
P. pygmeus	2	1 (50)	13
P. sulcirostris	4	2 (50)	13
P. urile	19	7 (37)	12
P. varius	9	1 (11)	12–13
ANHINGIDAE			
Anhinga	36	10 (28)	12
A. anhinga	24	6 (25)	12
A. melanogaster	1	0 (0)	—
A. novaehollandiae	3	1 (33)	12
A. rufa	8	3 (38)	12
SULIDAE			
Morus	48	20 (42)	12
M. bassanus	37	14 (38)	12
M. capensis	9	5 (56)	12
M. serrator	2	1 (50)	12
Sula	148	52 (35)	10
S. dactylatra	37	12 (32)	10
S. nebouxii	15	7 (47)	10
S. variegata	16	5 (31)	10
S. leucogaster	39	14 (36)	10
S. sula	41	14 (34)	10
Papasula	1	1 (100)	12
P. abbotti	1	1 (100)	12
Totals	695	295 (42)	

to the most recent common ancestor of the taxa included in the traditional Pelecaniformes, which they consider to be polyphyletic. These outgroup taxa also have either 14 or 15 scleral ossicles (Curtis and Miller 1938). Therefore 14 or 15 scleral ossicles appears to be the primitive condition not only for the Neognathae but also for the Pelecaniformes.

At least two reductions in ossicle number must have occurred within the Pelecaniformes. Furthermore, these reductions are congruent with the two current hypotheses of pelecaniform relationships (Figs. 1, 2). The traditional and widely accepted hypothesis (Fig. 1) has been supported cladistically by Cracraft (1985). *Phaethon, Fregata,* and *Pelecanus* retain the primitive number of 15 ossicles per ring, but the clade stemming from the most recent common ancestor of the sulids, cormorants, and anhingas (=Sulae Sharpe 1891) is derived in that the number of ossicles is reduced to 12 or 13. A further decrease in ossicle numbers occurs within the Sulidae; *Sula* has only 10 ossicles per ring (some specimens show as few as 8), compared with 12 in both *Morus* and *Papasula*. The 10 scleral ossicles per ring in *Sula* is the smallest number known for birds (see Lemmrich 1931, Curtis and Miller 1938).

These proposed transformations in numbers of ossicles is also congruent with an alternative

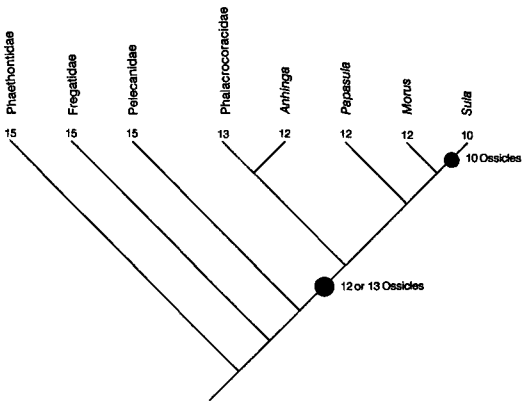

Fig. 1. Phylogenetic hypothesis based on Cracraft (1985), except relationships within the Sulidae (*Papasula, Morus,* and *Sula*) which are based on Olson and Warheit (1988). The number associated with each taxon represents the modal number of ossicles/scleral ring for that taxon.

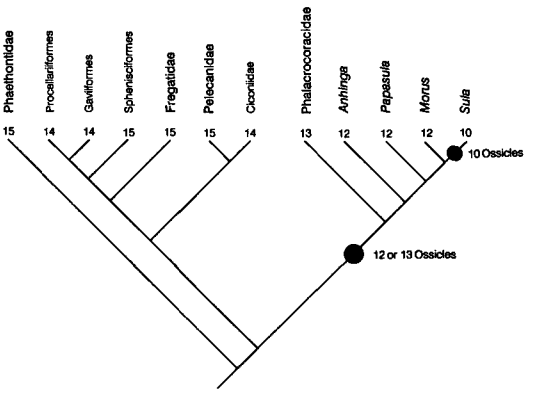

Fig. 2. Phylogenetic hypothesis based on Sibley et al. (1988). Threskiornithidae (15 ossicles [Lemmrich 1931]), Phoenicopteridae (14 ossicles [this study]), Scopidae (15 ossicles [this study]), and Ardeidae (14–15 ossicles [Lemmrich 1931, Curtis and Miller 1938]) are successive outgroups to the Ciconiidae-Procellariiformes clade and have been omitted to simplify the figure. Relationships within the Sulidae are based on Olson and Warheit (1988), and the number associated with each taxon represents the modal number of ossicles/scleral ring for that taxon.

hypothesis for the relationships of the "pelecaniforms" (Fig. 2). Sibley et al. (1988) proposed that the Pelecaniformes is polyphyletic, with Fregatidae and Pelecanidae more closely related to ciconiids, sphenisciforms, gaviiforms, and procellariiforms than to the Sulae (=Sulida in Sibley et al. [1988]). Nevertheless, because the Sulae is considered to be monophyletic and because its hypothesized outgroups all have 14 or 15 ossicles per ring, this hypothesis also implies reductions in ossicle numbers in the most recent common ancestor of Sulae and of *Sula*.

From the perspective of ossicle transformations, an important distinction between the traditional hypothesis and that of Sibley et al. (1988) concerns the relationships within the Sulae. Both hypotheses require the same number of ossicle transformations, yet the transformations themselves differ (Fig. 3). In the traditional hypothesis (Fig. 1), *Phalacrocorax* and *Anhinga* are sister taxa. This hypothesis requires at least three ossicle transformations: reduction to 12 ossicles for the Sulae, increase to 13 ossicles for *Phalacrocorax,* and reduction to 10 ossicles for *Sula* (Fig. 3a). An alternative hypothesis of ossicle evolution involving reduction to 13 ossicles for the Sulae, convergent reduction to 12 ossicles in *Anhinga* and Sulidae, and reduction to 10 ossicles for *Sula* requires at least four transformations (Fig. 3b). In the hypothesis of Sibley et al. (1988), *Anhinga* and the Sulidae are sister taxa (Fig. 2). These relationships also require at least three ossicle transformations: either reduction to 12 ossicles for the Sulae, increase to 13 ossicles for *Phalacrocorax,* and reduction to 10 ossicles for *Sula* (Fig. 3c); or, reduction to 13 ossicles for the Sulae, reduction to 12 ossicles for *Anhinga* plus the Sulidae, and reduction to 10 ossicles for *Sula* (Fig. 3d).

Of these four alternative hypotheses of ossicle evolution within Sulae (Fig. 3), the hypothesis represented by Figure 3b requires one more transformation than those represented by Figures 3a, 3c, and 3d. It is also the only alternative involving homoplasy, specifically convergent reduction from 13 to 12 ossicles in *Anhinga* and the Sulidae. Although the hypothesized transformations concerning *Phalacrocorax* in Figures 3a and 3c involve increases in ossicle numbers (from 12 to 13 ossicles), these need not be interpreted as homoplasies. Because there is no evidence that the common ancestor of the Sulae passed through a 13 ossicle stage (transformation could have been from 14 or 15 ossicles directly to 12 ossicles), an increase from 12 to 13 ossicles does not necessitate a character reversal.

Although the number of ossicles per ring var-

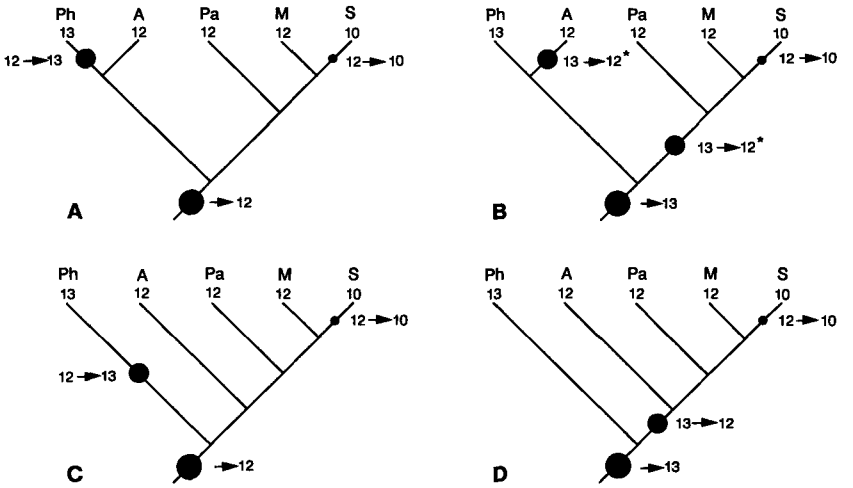

Fig. 3. Relationships within the Sulae, according to Cracraft (1985) (a, b) and Sibley et al. (1988) (c, d). Relationships within the Sulidae are based on Olson and Warheit (1988). Enclosed circles represent proposed scleral ossicle transformations (see text). Size of enclosed circles identifies specific points on the cladograms. The smallest circle represents the 12 to 10 ossicle transformation associated with the genus *Sula*; the largest circle represents the ancestral Sulae ossicle transformation (from the outgroup condition to either 12 or 13 ossicles). The medium-sized circle represents alternative transformations within the Sulae. Asterisk (*) indicates convergent transformations. S = *Sula*, M = *Morus*, Pa = *Papasula*, A = *Anhinga*, and Ph = Phalacrocoracidae. The number associated with each taxon represents the modal number of ossicles/scleral ring for that taxon.

ies among families and genera of pelecaniforms, it is relatively constant within genera. This conservative pattern of ossicle variability within genera should not be construed as being of phylogenetic significance because it depends on subjective taxonomic ranking. Although the gannets (*Morus*) always have been considered "taxonomically distinct" from the boobies (*Sula*), the two groups are often placed in a single genus. In the six editions of the A.O.U. check-list, gannets have alternately been considered either *Morus* or *Sula*, switching three times in 73 years (editions 3–6). Based on the most recent A.O.U. check-list (1983), in which all sulid species are included within the genus *Sula*, there would be intrageneric variation in modal ossicle number. If three sulid genera are recognized (Olson and Warheit 1988), however, there is no variation in modal ossicle number within each genus. The amount of variation within the Sulidae has not decreased, it is simply partitioned differently. A similar situation exists within the Phalacrocoracidae. *Phalacrocorax gaimardi, P. magellanicus, P. pelagicus, P. urile,* and *P. bougainvillii* have 12 (or 12–13) ossicles per ring, rather than the modal number of 13 present in the other Phalacrocoracidae. Siegel-Causey (1988) placed *P. gaimardi, P. magellanicus, P. pelagicus,* and *P. urile* in the genus *Stictocarbo.* As a result, the intrageneric variation in ossicle number within the cormorants is decreased. *P. bougainvillii* is hypothesized to be convergent with *Stictocarbo* in possessing 12 ossicles per ring. Although Siegel-Causey (1988) did not use modal number of ossicles in his analysis, their distribution within the Phalacrocoracidae is largely congruent with the phylogeny he proposed.

Reductions in scleral ossicle number are derived and diagnostic for the Sulae, *Sula,* and *Stictocarbo* within Pelecaniformes. Derived reductions in ossicle numbers also occur in several distantly related taxa. For example, within the Charadriiformes, the Alcidae are derived in having 13 scleral ossicles compared with 15 ossicles for most other charadriiforms (Curtis and Miller 1938). Other avian taxa characterized by 11 or 12 scleral ossicles are Columbiformes, Psittaciformes, and Cuculiformes (Curtis and Miller 1938, de Queiroz and Good 1988). The scleral ring has been investigated in detail for

very few groups, however, and more work is necessary to reach an understanding of the evolution of this character within birds.

From our experience, a comparative study of ossicle variation at any level within the avian hierarchy would be difficult to accomplish, because the scleral rings of different taxa are not always well represented in museum collections. We found that there are more specimens of *Fregata* and *Phaethon*, and fewer of *Pelecanus* in museum collections that include scleral rings, than would be expected from a random distribution. Because of the potential systematic importance of scleral rings, we recommend that museums increase their efforts to preserve these elements. If a museum uses maceration to clean skeletons, the eyes should be removed beforehand. Maceration can still be used to prepare the isolated ring, but it must be checked frequently to prevent disarticulation. If the skeleton is prepared by mechanical means, we recommend that the eyes not be removed; beetle larvae are capable of cleaning scleral rings. Nevertheless, when removing the prepared skeleton from the beetle frass, special attention must be paid to scleral rings and other small elements. The larvae themselves may complicate the process by removing and transporting rings. This occurs most frequently with small rings when the larvae become trapped within the center of the ring, wearing it as if it were a life-preserver.

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