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RETINAL MORPHOLOGY AND ELECTRORETINOGRAPHY IN TWO VISUALLY FORAGING CHARADRIIFORMES BIRDS WITH DIFFERENT FEEDING ACTIVITY RHYTHMS: THE DOUBLE-STRIPED THICK-KNEE (*BURHINUS BISTRIATUS* WAGLER, 1829) AND THE SOUTHERN LAPWING (*VANELLUS CHILENSIS* L., 1758)

MORFOLOGÍA Y ELECTRORRETINOGRAFÍA RETINIANA EN DOS AVES CHARADRIIFORMES CON DIFERENTES RITMOS VISUALES DE ALIMENTACIÓN: LA DARA (*BURHINUS BISTRIATUS* WAGLER, 1829) Y EL ALCARAVÁN (*VANELLUS CHILENSIS* L., 1758)

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ABSTRACT

Our study compares the visual function of the Double-striped Thick-knee (*Burhinus bistriatus* Wagler, 1829), which forages primarily during dusk and at night, and the Southern Lapwing (*Vanellus chilensis* L., 1758), which is known to forage during daytime and occasionally at night, analyzing morphological and electrophysiological aspects of their retina. The fact that thick-knees have large eyes and are nocturnally actives suggest that, compared with the diurnal lapwing, they should have a very sensitive retina under low light intensity. Electroretinograms (ERGs) were obtained from anesthetized live birds at different light intensities in photopic and scotopic conditions, and the retinae were subsequently processed for histological analysis. The scotopic ERG *b*-waves of *B. bistriatus*, at all light intensities, were always of larger amplitude than those of *V. chilensis*. However, the *a*-waves of both species were of similar amplitude. Under photopic conditions, *V. chilensis* yield highest *a*- and *b*-wave amplitudes than *B. bistriatus*. The latter has a larger dialated pupil diameter and a greater axial length/equatorial diameter ratio than *V. chilensis*. Likewise, the rod density of *B. bistriatus* significantly exceeds that of *V. chilensis*. In the latter, cone density tends to be higher than in *B. bistriatus* while the rods:cones ratio were lower. Rod outer segments of *B. bistriatus* strongly exceed in length those of any other Charadriiformes species studied so far, but are thinner than those of *V. chilensis*. In contrast, the latter has thicker cone outer segments and outer and inner plexiform layers than *B. bistriatus*. Similarly, ganglion cells are more abundant per unit area in *V. chilensis*. Our combined results reveal a higher retinal sensitivity of *B. bistriatus* under low light conditions, in accordance with their crepuscular and nocturnal foraging strategies. *V. chilensis*, although mainly active during daylight, appears to have a moderate retinal sensitivity under low light conditions. Thus, we conclude that Southern Lapwings present visual adaptations that enable them to function in nocturnal as well as in diurnal conditions.

Key words: *Burhinus bistriatus*, electroretinography, retinal morphology, *Vanellus chilensis.*

RESUMEN

Se comparó la función visual de dos aves terrestres Charadriiformes: la dara (*Burhinus bistriatus* Wagler 1829), de hábitos de crepusculares y nocturnos, y el alcaraván (*Vanellus chilensis* L. 1758), primordialmente diurnos y ocasionalmente nocturnos, utilizando electrorretinogramas (ERG), mediante análisis morfológicos y electrofisiológicos de sus retinas. Dado que las daras tienen los ojos relativamente más grandes y mayor actividad nocturna que los alcaravanes se esperarían que tuvieran una retina más sensible bajo condiciones de escasa iluminación. Las aves fueron anestesiadas para obtener los electrorretinogramas bajo condiciones escotópicas y fotópicas y luego sacrificadas para el análisis histológico de la retina. A todas las intensidades luminosas probadas, las ondas *b* escotópicas fueron siempre de mayor amplitud en *B. bistriatus* que en *V. chilensis*; no obstante, la ondas *a* fueron similares en ambas especies. Bajo condiciones fotópicas, *V. chilensis* produjo ondas *a* y *b* de mayor amplitud que *B. bistriatus*. El diámetro de la pupila dilatada y la proporción longitud axial/diámetro ecuatorial de *B. bistriatus* fue mayor que en *V. chilensis*. Por su parte, la densidad de bastones fue significativamente superior en *B. bistriatus* que en *V. chilensis*; en cambio, la densidad de conos tendió a ser más alta en *V. chilensis*, aunque menor la proporción bastones:conos. Los segmentos externos de los bastones de *B. bistriatus* superaron visiblemente en longitud a los de cualquier otra especie de caradriforme estudiada hasta ahora, aunque son más delgados que los de *V. chilensis*. En contraste, los segmentos externos de los conos y las membranas plexiforme externa e interna son más gruesas en *V. chilensis*. De igual modo, en *V. chilensis*, las células ganglionares fueron más abundantes por unidad de área. Nuestros resultados revelan que *B. bistriatus* tiene una alta sensibilidad retiniana en condiciones de baja luminosidad, en sintonía con su estrategias alimentarias crepusculares y nocturnas. *V. chilensis*, a pesar de estar activo mayoritariamente de día, parece tener una moderada sensibilidad retiniana en condiciones de baja luminosidad, concluyendo, entonces, que los alcaravanes presentan adaptaciones visuales que los capacitan para funcionar tanto en condiciones diurnas como nocturnas.

Palabras clave: *Burhinus bistriatus*, electrorretinografía, morfología retiniana, *Vanellus chilensis.*

INTRODUCTION

"Light and the light/dark cycles were probably the most important selective forces ever to have ever acted on biological organisms", and consequently, "one of the most remarkable consequences of light on earth has been the evolution of vision" (Fernald 1997). Indeed, it is generally believed that "differences in gross anatomy reflect adaptations of the basic structure of vertebrate eyes to differences in the visual problems imposed by different life styles" (Martin & Brooke 1991), and it "might be expected that the eyes of nocturnally active species would have lower minimum *f*-numbers (higher maximum image brightness) than diurnal species" (Martin 1994). Furthermore, nocturnally active species tend to have larger and globular eyes, i.e., higher axial length/equatorial diameter (AL/ED) ratios, than diurnal species (Martin 1990, Waldvogel 1990).

Nocturnality, the habit of being active between sunset and sunrise, has been viewed as a characteristic of a minority of bird species, e.g., Apterygiformes, Strigiformes, Caprimulgiformes, Apodiformes (Martin 1990). However, while strict nocturnality is rare, e.g., oilbird (*Steatornis caripensis* Humboldt, 1814) (Rojas *et al.* 2004, Martin *et al.* 2004), studies in the last decade have shown that nocturnal activity is widespread among marine, freshwater, shore and marsh birds, and occurs in members of 9 orders and 28 families of waterbirds (McNeil *et al.* 1992, 1993).

Comparative ecophysiological studies have been made in several Charadriiformes aquatic birds belonging to the families Rynchopidae (Rojas *et al.* 1997), Scolopacidae, Charadriidae, Recurvirostridae (Rojas *et al.* 1999a), as well as in Ardeidae and Threskiornithidae (Rojas *et al.* 1999b), mainly analyzing visual capacity and their ecological significance. Of these studies, we can

conclude that the retinal structure shorebird show a direct relationship with their periods within the daily light cycle in which they forage.

Thick-knees (Burhinidae) and lapwings (Vanellinae) are terrestrial shorebirds whose nocturnal activities have been documented by direct observation only on rare occasions (e.g., Milssom 1984, Milssom *et al.* 1990, Solís & de Lope 1995, Thomas 1999). Thick-knees live in dry, open country usually away from water habitats. Double-stripped Thick-knees (*Burhinus bistriatus* Wagler, 1829) are sometimes fairly common but are difficult to locate; during daytime, they rest in sun or shady place and crouch to avoid detection (Hilty 2003). In Venezuela they are widespread to 200 m but less numerous and local in arid scrub near coast (Hilty 2003). Double-striped Thick-knees seem to have foraging activities exclusively at dusk and night; and exceptionally during cloudy and rainy days (Del Hoyo *et al*. 1996). In contrast, Southern Lapwings (*Vanellus chilensis* L. 1758) forages largely by day, and seem to have activities occasionally at dusk and night. They are noisy birds, usually found in pairbonds, families or seasonally in groups in open and short pastures, and occasionally in marsh areas.

Our study compares both morphological and electrophysiological aspects of the retinas of Double-striped Thick-knees and Southern Lapwings. Both species typically forage using visual searching, scanning an area while still standing, before running to peck at prey (Del Hoyo *et al.* 1996). There is no information about the visual capacities of these two species; nevertheless, the fact that thick-knees have large eyes and are nocturnally actives suggests that, compared with the diurnal lapwing, they should have a very sensitive retina under low light intensity. Accordingly, the purpose of this study was to compare some morphological and functional aspects of the retinas of both species, and examine how they correlate with their periods of foraging activity. The results will also be compared to those from obtained in other species using the same research protocol.

METHODOLOGY

The birds were obtained under licence from the Ministerio del Ambiente of Venezuela (Ley de Protección a la Fauna Silvestre: Tít. I-Cap. 1- Art. 6/Tít. II-Cap. 1-Art.14). Individuals were spot-lighted after dawn and caught by hand with the use of throw-nets or mist netted, in the Mapire region (state of Anzoátegui) of the Venezuelan "llanos".

ERG recording

The electroretinogram (ERG) is the recording of electrical potentials produced by the retina in response to a light stimulus, and which can be recorded at a distance, i.e., at the cornea [for review, see Ikeda 1993]. A typical ERG consists of two waves which arise in different layers of the retina, reflecting light-evoked potentials generated by different retinal cells (Dick & Miller 1985). The first one (*a*-wave), negative, is generated mainly by the photoreceptors; the second one (*b*-wave), positive, takes origin in the inner nuclear layer (Armington 1974). The waveforms of the ERG and its components exhibit changes depending on the intensity and wavelength of the stimulating flash, as well as the state of retinal adaptation, i.e., photopic (cone-mediated) and scotopic (rod-mediated).

The number of birds used for ERG recording was four for each of the two species studied. The birds were brought alive to the laboratory at the Universidad de Oriente, in Cumaná (Venezuela), or to a field camp nearby the site where they were captured. ERGs were recorded in a dark room with the use of a LKC EPIC-2000 visual electrodiagnostic system (LKC Technologies Inc., Gaithersburg, MD), which includes a 41-cm diameter full field stimulator (LKC Ganzfeld-2503B), using a method previously reported (Rojas *et al*. 1997, 1999a,b). Briefly, after a period of 4 h of dark adaptation to allow for the recording of scotopic responses, the birds were anesthetized with a 1:1 mixture of ketamine-xylazine (0.0044 cc/kg injected in the pectoral muscle), and immobilized on a home-made recording holder with the head kept inside the stimulator and the left eye maintained open upward.

The left eyelids and nictitating membrane were kept retracted with a speculum, the cornea was anesthetized with 0.5% proparacaine hydrochloride, and the pupil was dilated with 1% tropicamide. The maximum pupil diameter (mm) was measured at the beginning and at the end of the experiment.

The active electrode consisted of a DTL® fiber (Sauquoit Industries, Scranton, PA) which was placed on the cornea (Lachapelle *et al*. 1993, Hébert *et al*. 1996). Subdermal needles (Grass Instruments, Astro-med Inc., Warwick, RI), inserted under the skin of the crown and in the pectoral muscle, served as reference and ground electrodes, respectively. ERG responses (average of 6 at 10.1-sec intervals) were evoked to flashes of increasing luminance of -4.8, -3.8, -3.0, -2.6, -2.0, -1.4, -1.0, and 0.0 log units of attenuation (maximal intensity: 3.31 cd m^{-2} sec^{-1} or 0.519 log cd sec^{-1} m^{-2}). Flash duration was 20 miliseconds (ms). The birds were then light-adapted for 10 min to a background luminance of 35.7 cd·m^{-2}, following which the photopic ERGs (average of 10 at 4.1-sec intervals) were evoked to flashes of decreasing luminance (0.0, -0.6, -1.0, and -1.4, -4.8 log units; maximal intensity: 3.31 cd m^{-2} sec^{-1}). Previous studies indicated that the above parameters resulted in adequate and reproducible segregation of rod and cone functions in birds (Rojas *et al*. 1997, 1999a,b). To facilitate comparison of data between species of birds, luminance-response function curves were generated from the scotopic ERG responses (Naka & Rushton 1966). We calculate the intensity of the stimulus necessary to evoke a b-wave whose amplitude is half (1/2 Vmax) the saturate amplitude (Vmax) of the response evoke by a very bright flash in scotopic condition. This value is generally considered as an adequate measurement of retinal sensitivity under scotopic condición [see Massof *et al*. 1984, Fulton 1991].

Histological preparation

Once the ERG recordings were completed, individuals of each species were kept for histological analysis and were euthanized under anesthesia. The left eye was removed and the axial length and equatorial diameter were measured (see Martin 1986).

The eye was then injected with 2.5% glutaraldehyde in 0.1 M phosphate buffer pH 7.4, punctured at the cornea, and placed in the same fixative for 45 min. Working with the eye in the fixative, the anterior part of the eye was removed and the retina, still attached to the choroid, was cut into 9 sectors, using the pecten as landmark.

This division is the same as that used by Rojas et al. (1997, 1999a,b), and corresponds to that of Meyer and May (1973) and Begin and Handford (1987), although the sector numbering is different. Still in the fixative, each sector was subdivided into 2-mm^2 portions, of which two were retained for analysis. After 45 min in the fixative, the retinal portions, separated from the choroid, were washed in 0.1 M phosphate buffer for 15 min, postfixed in 1% OsO_4 in 0.1 M phosphate buffer for 1 h, rinsed in phosphate buffer followed by two baths in distilled water (10 min each), dehydrated in graded ethanol (from 50% to 100%, 5 min per step), and bathed in propylene oxide (10 min).

The tissues were successively infiltrated with a 2:1 mixture of Epon and propylene oxide for 6 h, and pure Epon-812 medium for another 2 h. Finally, they were embedded in silicone rubber molds filled with Epon-812 and polymerized at 60°C for 48 h.

Semithin (0.7 µm) sections were obtained and 15 of them (one every 30-40 sections) were mounted on glass slides and stained with toluidin blue. Cuts were made perpendicularly to the retina by reorienting the blocks until achieving sections longitudinal to the photoreceptors.

Data analysis

The analysis of morphological measurements included the mean calculations (± 95% confidence intervals) of the dilated pupil diameter, cell densities (rod, cones, and ganglion cells), length and diameter of the outer segment of rods and cones, and the thickness of each retinal layer were measured. Rods, cones and ganglion cells were counted in 238-µm wide fields, for a total of 15 counts for each of the nine sectors. As in other avian retinae (Meyer & May 1973, Meyer 1977, Tansley & Erichsen 1985, Waldvogel 1990), double cones, in addition to single cones, were present in both species, and they were

counted as two cones. Ganglion cells were identified according to morphology and coloration criteria (Hayes & Brooke 1990, Inzunza *et al*. 1991) and counted.

To estimate the minimum *f*-number (f_{min}) and the relative image brightness of the two species, we use the following equations (Thomas *et al*. 2004): f_{min} = Focal length/maximum pupil aperture, where focal length is estimated to be approximately equal to 0.6 x axial length. This is a measure of the maximum light gathering capacity of an optical system when viewing extended light source.

Statistical treatment

The species were compared by utilising a conventional statistical analysis, the 95% confidence interval on the means. Additionally, in ERGs obtained in scotopic and photopic conditions, we used the Mann-Whitney U-test for unmatched pairs with smaller numbers of sampling, and the Wilcoxon's test for matched pairs when sample units > 15. In histological analysis, when the number of sampling units in either sample was 25 we used the *t*-test for differences between means (Fowler & Cohen 1996).

RESULTS

Electroretinography

Representative ERGs obtained in scotopic and photopic conditions for each species are presented in Figure 1. It can be seen that the generated waveforms (*b*-wave and *a*-wave) differed in shape and amplitude between Double-striped Thick-knees and Southern Lapwings.

Scotopic and photopic conditions

In general, Double-striped Thick-knees showed mean scotopic b-wave amplitude higher than that of Southern Lapwings (Figure 2-A). Effectively, as shown by the 95% confidence intervals, *b*-wave amplitude of Double-striped Thick-knees increased nearly linearly at lower luminance intensities (-5.0 to –3.0 log units), and was followed by a saturation phase (with slight increases and decreases), tending to be significantly higher than that of Southern Lapwings (TW = 79 P < 0.05). In contrast, scotopic a wave amplitudes of both species were about the same and varied similarly, and more or less exponentially as a function of luminance intensity (Figure 2-B) (t= 2,60 > 2.06, P = 0.05).

In contrast, under photopic conditions, Southern Lapwings tended to yield b-wave amplitudes higher than Double-striped Thick-knees (Figure 3-A y 3-B) but the differences were significant only at highest light intensity (UM-W =17 P > 0.05), while a-wave amplitude showed similar patterns from each other(UM-W =3 P > 0.05).

The intensity of the stimulus necessary to elicit 1/2Vmax in scotopic condition varies slightly in bouth species analyzed. Contrary to expectations, Southern Lapwings have a sensitivity hardly higher than Double-striped Thick-knees (-4.3 vs -4.0 log. units of light attenuation).

Morphological results

Morphological results for each species are presented in Table 1. Double-striped Thick-knees had a larger eye, a greater pupillar diameter (9 mm vs. 7 mm) and axial lenght:equatorial diameter ratio (0.81 vs. 0.70) than the Southern Lapwings. Double-striped Thick-knees had a higher f-number than the Southern Lapwings (*f*-number: 1.96 vs. 1.24).

Histological data

A photomicrograph from S_5 of the retina in both species is shown in figures 4-A and 4-B. Retinae of both species differed in photoreceptors density and thickness layers. Based on the 95% confidence intervals, the rod density in Double-striped Thick-knees significantly exceeded that in Southern Lapwings (*t*= 7.02 > 2.97, *P* = 0.01, d.g.14), with overall rods:cones ratio 3:1 vs. 0.7:1 (Table 1). In Double-striped Thick-knees, rod density (51,62 ± 4,95 vs. 36,87 ± 2,58) and rod:cone ratio tended to be higher in all sectors; particularly, rods tended to be significantly more numerous in central sector (S_5) than in other sectors.

Double-striped Thick-knees showed lower cones density than Southern Lapwings (17,87 ± 1,80 vs. 52,87 ± 3,83), and their cones density tended to be lower in sectors 6, 7 and 9 (Figure 4A). On the average, Double-striped Thick-knees had markedly longer (64.75 ± 2.60μm vs. 44.38 ± 1.76 μm) but thinner (3.75 ± 0.46 μm vs. 4.98 ± 0.07μm) outer rod segments than Southern Lapwings (*t*= d.g.14, 5,22 > 2.97, *P* = 0.01); in fact, they significantly exceeded that the of all other Charadriiformes species yet studied (Table 1).

In contrast, Southern Lapwings had a clearly cone-dominated retina (Figure 5 and 4-B). Cone density tended to be significantly higher in S_2, S_4, S_5, S_7 and S_9. Southern Lapwings had longer (20.0 ± 1.92μm vs 16.5 ± 1.51 μm) and thicker (2.98 ± 0.04μm vs 1.00 ± 0.03 μm) cone outer segment than Double-striped Thick-knees (*t*= 3,80 > 2.97, *P* = 0.01, d.g.14). Similarly, ganglion cell number was higher in Southern Lapwings, particularly in S_5 which presented a double row of ganglion cells (Figures 6-A and 6-B). In both species, photoreceptors tend to be more numerous in S_5 than in others sectors (Figures 4-A and 4-B).

It can be seen that the thickness of the inner nuclear (75.00 ± 5.97 μm vs 51.75 ± 2.31 μm) and inner plexiform (90.62 ± 1.76 μm vs 69.25 ± 1.75 μm) layers, as well as the density of ganglion cells (Table 1), tends to be greater in Southern Lapwings compared with Double-striped Thick-knees (Figures 5 and 6A-6B).

DISCUSSION

Our combined histological and electrophysiological results show that the retinas of the two studied species are structured and respond differently for night and for day vision, in accordance with their foraging strategy and periods of foraging activity. Both species are visually guided peckers, the Double-striped Thick-knee exclusively at dusk and at night, and the Southern Lapwing typically by day and occasionally at dusk and night.

Double-striped Thick-knee and Southern Lapwing have a scotopic sensitivity higher than the others Charadriiformes species studied yet (Rojas *et al.* 1999a).

The higher scotopic sensitivity of Double-striped Thick-knee can result from their very long rod outer segments; in fact, they significantly exceeded that of all other Charadriiformes analyzed (Tabla 1). In addition, their retina is highly rod-dominated (3:1) and they have a large pupil diameter (9 mm). Indeed, their ED exceed that measured for the other Charadriiformes species (Rojas *et al.* 1999a); nevertheless, is smaller than that of the Yellow-crowned Night Heron (*Nycticorax violaceus* L., 1758) a crepuscular and nocturnal sight feeder (Rojas *et al.* 1999b).

Likewise, their AL:ED ratios are lower than those of the Wilson's Plover (*Charadrius wilsonia* Ord, 1814), a visual pecker which appear to be well adapted for nocturnal vision; the Black-winged Stilt (*Himantopus himantopus* Müller, 1776) and the Willet (*Catoptrophorus semipalmatus* Gmelin, 1789), shorebirds known to forage for visual pecking at moonlit nights (Rojas *et al.* 1999a); as well as the Black Skimmer (*Rynchops niger* L., 1758), known to forage in flight using tactile cues, and only at dusk and night (Rojas *et al*. 1997).

The fact that *f*-number value (1.11) in Southern Lapwing is lower compared to Double-striped Thick-knee (1.40) means that when viewing the same scene the Southern Lapwing eye retinal image will be brighter than Double-striped Thick-knee. This finding should explain the observations of Milsson *et al.* (1990) who described the relationship between the diel activity patterns of the Northern Lapwing (*Vanellus vanellus* L., 1758) and the lunar cycle. They found that Northern Lapwing are not responding to a diel variation in prey availability at all but are forced to feed nocturnally, when moonlight levels permit because they are unable to balance their energy budget by feeding solely during

daylight and/or it be active at night under moonlight and to roost during daylight when mammalian predators are less active. In effect, at night its ingestion rates were considered approximately twice as high as those achieved during day (McLennan 1979).

In Argentine, Gallegos (1984) indicates that Southern Lapwing displayed activity during full moon. Likewise, we observed that during the breeding season, Southern Lapwing congregates in "leks" and display flights and sing shrilly at dusk and night.

Rojas *et al.* (1997) have showed that the increased sensitivity exhibited by the Black Skimmer is probably not the result only of their high rod density, high rods:cones ratio, nor of exceptionally long rod outer segments, but of the greater area covered by rods. In addition, rod sensitivity seems not solely determined by their length or diameter, but also by the density of the visual pigment they contain. In Oilbird (*Steatornis caripensis* Humboldt, 1817), for example, the high rod density and extremely high rod:cone ratio (123:1), and low *f*-numbers, have combined to provide this species with the most sensitive bird eye so far described (Rojas *et al*. 2004, Martin *et al*. 2004).

Under photopic conditions, Southern Lapwing tended to yield *b*-wave amplitudes higher than Double-striped Thick-knees (Figure 3-A y 3-B) but the differences were significant only at highest light intensity, while *a*-wave amplitude showed similar patterns from each other. Although in the Southern Lapwing cone density was roughly 3,5 times higher than in the Double-striped Thick-knee, the overall dimensions of Southern Lapwing eyes are considerably smaller than Double-stripedThick-knee. This should be the main reason in similar photopic responses in these species.

The Southern Lapwing, taken its higher cone density with its double row of ganglion cells and the thickness of its inner nuclear and inner plexiform layers, especially in the central sector, is well adapted for daytime visually-guided behavior, it should favour the panoramic vision (Rojas *et al*. 1999a, b). Increased ganglion cells density is usually found in the retinal regions subserving higher visual acuity (Inzunza 1991).

Rather, complex processing such as movement and direction selectivity occurs in the inner plexiform layer (Dowling 1987), then thicker inner plexiform layer and more ganglion cells density could result in increased responses of the retina and thus should provide a better vision of contrasts and prey and predator movements. Usually, Southern Lapwing forage by visual searching technique, scanning an area while standing still, before running forward rapidly to peck at prey (Del Hoyo *et al.* 1996). This species feeds on insects and other small invertebrates (beetles, ants, Diptera, crickets, grasshoppers, dragonflies, mostly in open seasonally floody grasslands near wetlands (Caballero *et al*. 2007).

Indeed, there is a trend in visually pecking species like the Wilson's Plover and the Willet to have a higher photoreceptor density, particularly of the cones, and a greater thickness of the inner nuclear, and inner plexiform layers, as well as ganglion cell density in the central retina (Rojas *et al.* 1999a). In general, all layers tend to be thinner in Double-striped Thick-knee, the largely nocturnal species, compared to those in Southern Lapwing, the "largely diurnal" species.

The higher sensitivity of Southern Lapwing than Double-striped Thick-knees was unexpected and surprising. Indeed, our results suggest that rods preponderance and higher rod outer segment length in the retina per se is not primarily related to sensitivity of Southern Lapwing.

Probably by having higher photoreceptors density in all retinal sector analyzed (Fig. 4) and rod:cone ratio lower (0.7:1 vs 3:1) but more evenly distributed, the Southern Lapwings' retina can be more efficient to the different light levels, hence enhancing overall sensitivity.

The lower rod density in Southern Lapwings seems in a way to be compensated by thicker rod outer segments. On the other hand, although having a relatively low rod density, a lower rods:cones ratio and lower scotopic ERG *b*-wave luminance-response function compared to Double-striped Thick-knees, Southern Lapwings present a scotopic ERG *b*-wave which reveals a comparable retinal sensitivity under low light conditions. It is possible that Southern Lapwing yields a higher ERG responses due to it has a mechanism of communication between photoreceptors well equipped, hence a more efficient neural circuitry, evidenced in the thicker inner plexiform layer than Double-striped Thick-knee (Fig. 6). All these combined features might enable to Southern Lapwing to yield a greater responses for a wider range of light intensities.

In addition, Southern Lapwing minimum *f*-number value (1.11) reveals that optically their eyes can performs higher light gathering capacity than the Thick-knee. Therefore, seemingly, the fact that Southern Lapwing is less active at night does not appear to result from poor night vision capability. Thus, we would conclude that Southern Lapwing presents visual adaptations that enable them to function in nocturnal as well as in diurnal conditions.

In any event, although optical design in birds appear to be one of the most remarkable selective modifications since feeding ecology is considered (Martin 2009), our results evidence that retinal structure and function result evolutionary primary forces in birds with diurnal and nocturnal feeding strategies, independently of their phylogenetical kinship (Gehring & Ikeo 1999, Ericson *et al*. 2003); therefore, in accordance with Archer *et al.* (1999), the balance of the features in any one eye would be achieved through adaptation of both optical and retinal structures, and is assumed to reflect both behaviour and ecology of the species.

Double-striped Thick-knee, although foraging mainly at dusk and night, and Southern Lapwing, although functioning in nocturnal as well as in diurnal conditions, and descending from a common ancestor (Ericson *et al*. 2003); nevertheless, taking into account the retinal morphological designs, and visual electrophysiological responses, these two Charadriiformes seem to have taken divergent behavioural and ecoevolutionary routes for exploiting their open habitats.

Finally, our results, in accordance with Fernald (1997), highlight the difficulty in analyzing the evolution of different eyes types, in dealing with structural similarity. In any case, two bird species could have a similar rod density mainly because they are closely related (McNeil *et al.* 1999).

Nevertheless, hierarchical relationships may lead to an untrustworthy perception by using conventional statistical tests (Garland *et al.* 1993). Inter-specific differences in retinal structural designs (e.g., rod-cone outer segment thickness and length, *f*-number value) and electrophysiological responses (i.e., ERGs) in Southern Lapwing and Double-stripped Thick-knee seem to demonstrate this difficulty.

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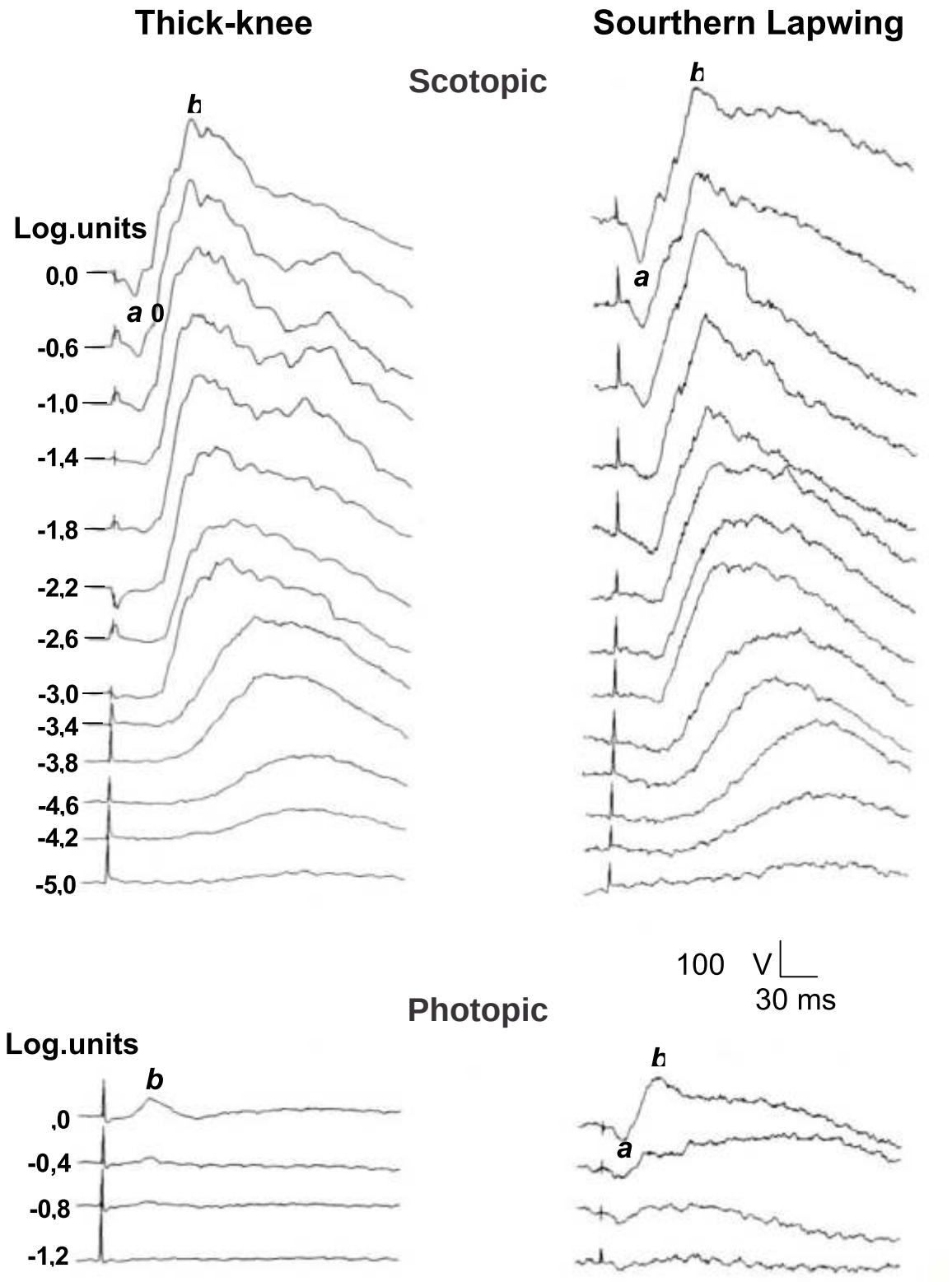

Figure 1. Representative ERG response of species studied, obtained of scotopic and photophic conditions. Nomenclature: a, peak of the *a*-wave; b, peak of the *b*-wave. The figures on the left represent light intensity values (Log units).

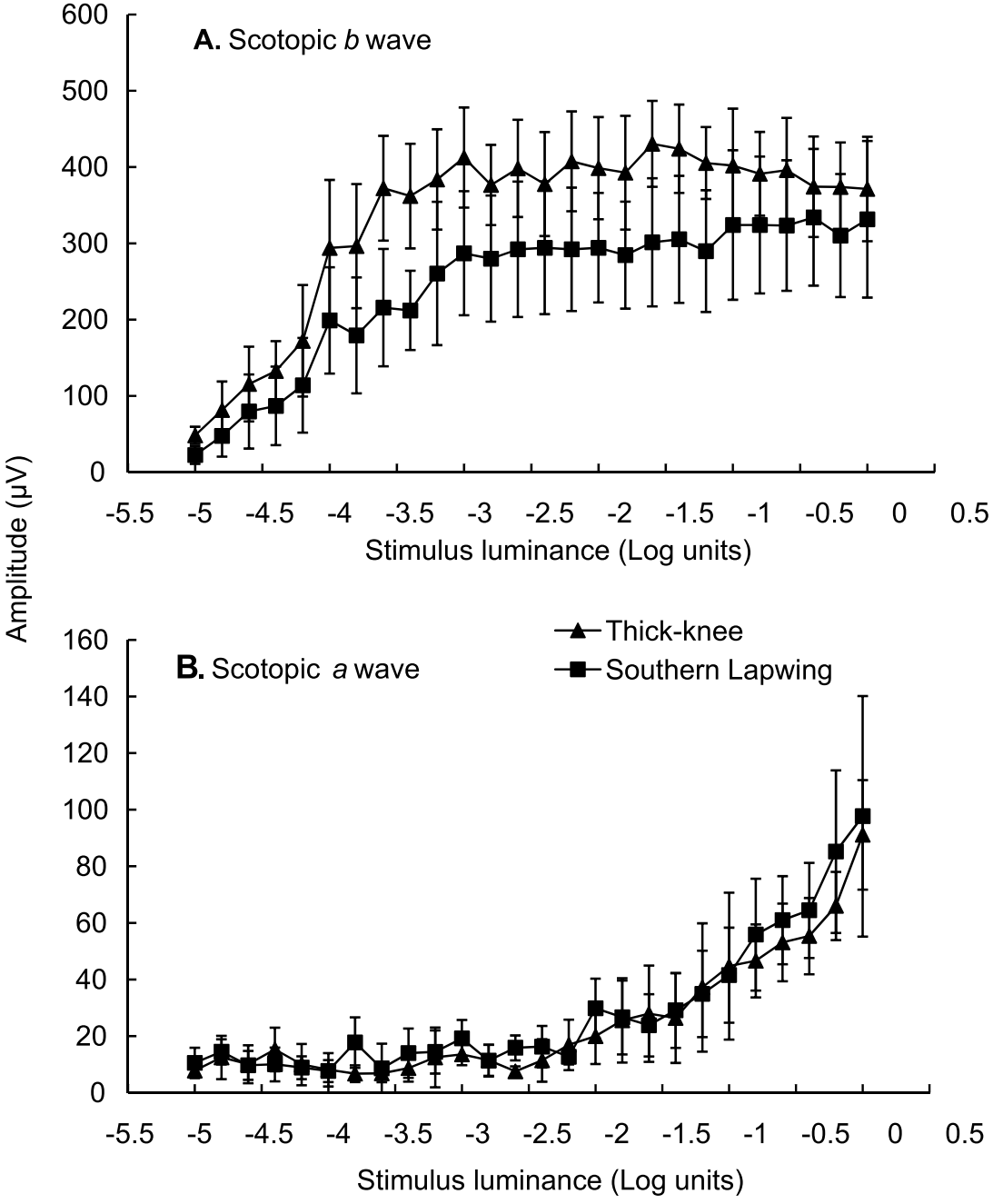

Figure 2. Luminance-response function (mean ± 95% confidence interval) of the *b*-wave of the species studied under scotopic (A) and photopic (B) conditions. The ordinate represents the *b*-wave amplitude (µV) and the abscissa the stimulus luminance intensity (Log units).

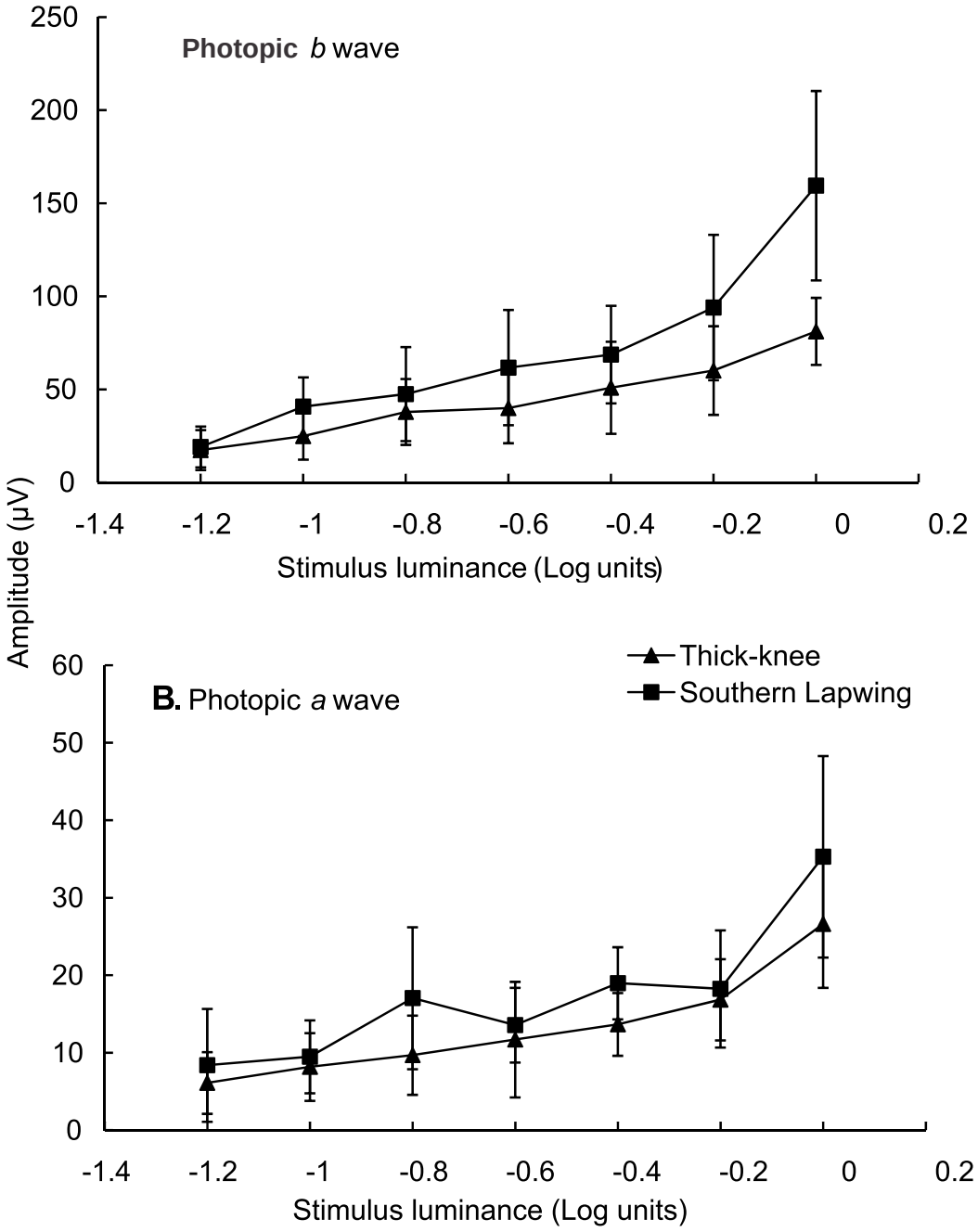

Figure 3. Luminance-response function (mean ±95% CI) of the *a*-wave of the species studied under scotopic (A) and photopic (B) conditions. The ordinate represents the *a*-wave amplitude (μV) and the abscissa the stimulus luminance intensity (Log units).

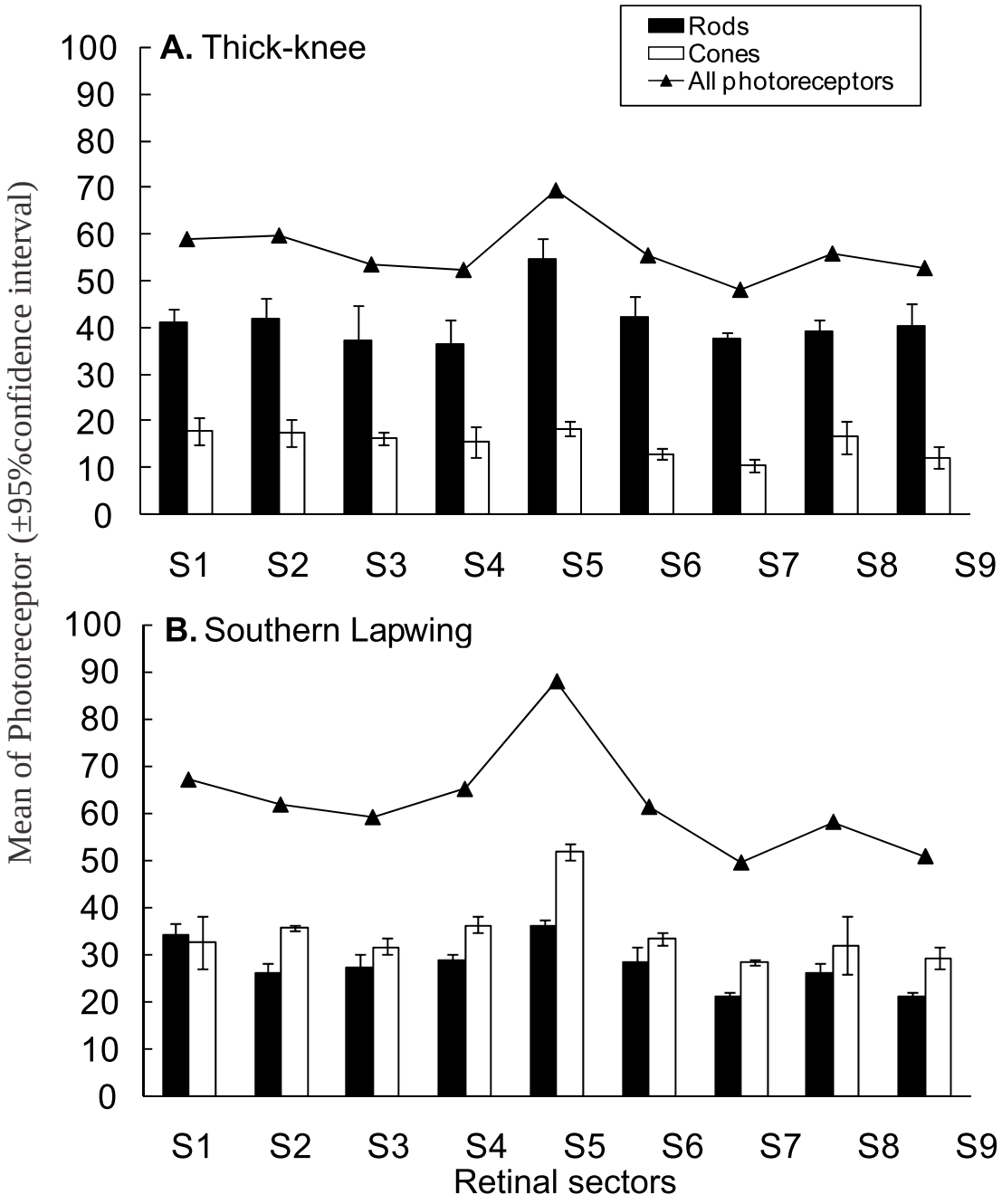

Figure 4. Mean rod and cone numbers per 238µm (±95% CI) of the species studied in each of the nine retinal sectors.

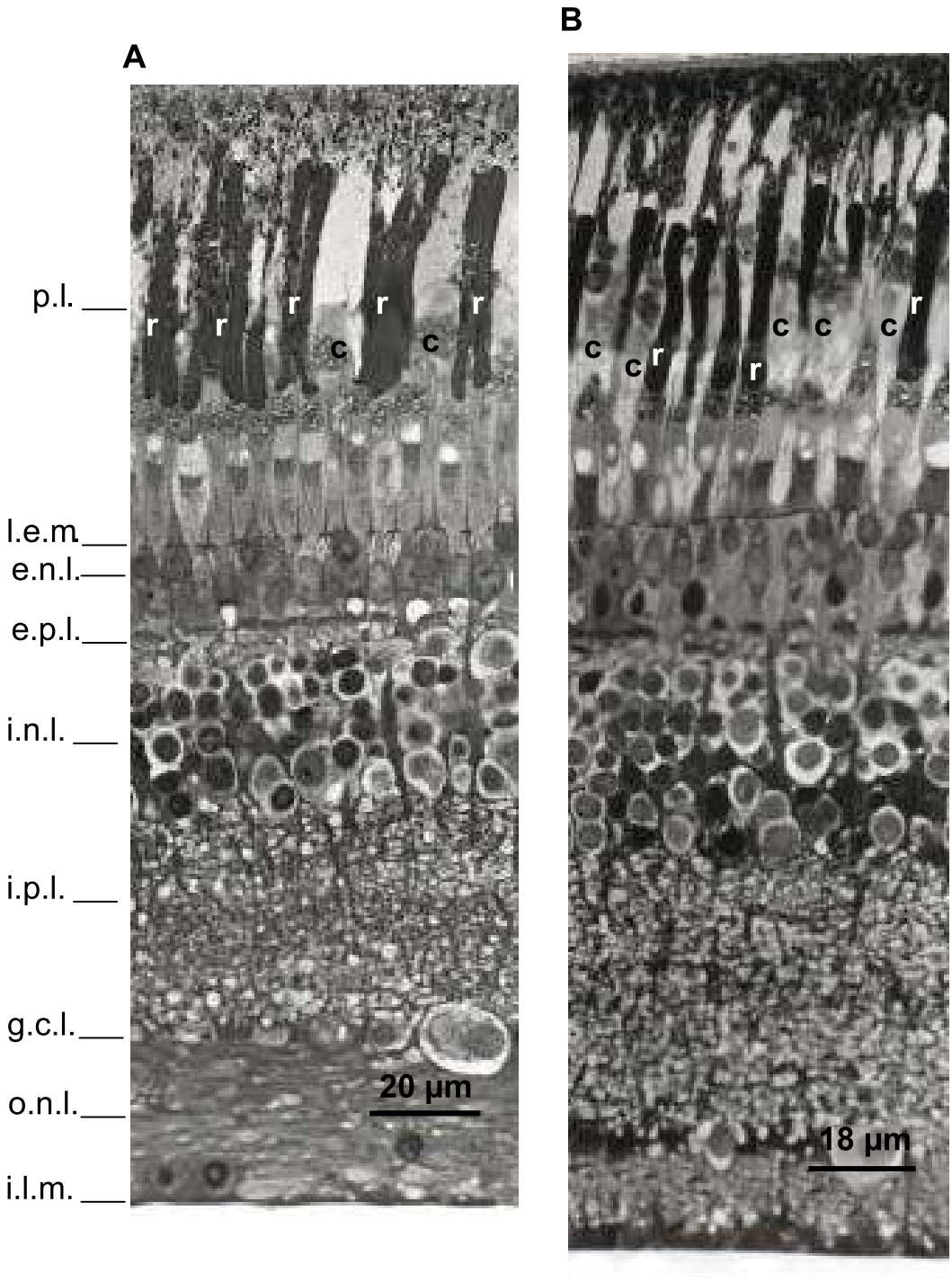

Figure 5. Photomicrographs showing the layers of sector 5 of the retina of the species studied. Nomenclature: c, cone; r, rods; e.l.m, external limiting membrane; g.c.l., ganglion cell layer; i.l.m., inner limiting membrane; i.n.l., inner nuclear layer; o.f.l., outer fiber layer; o.n.l.; outer nuclear layer; o.p.l., outer plexiform layer; p.l. photoreceptor layer; r: rods.

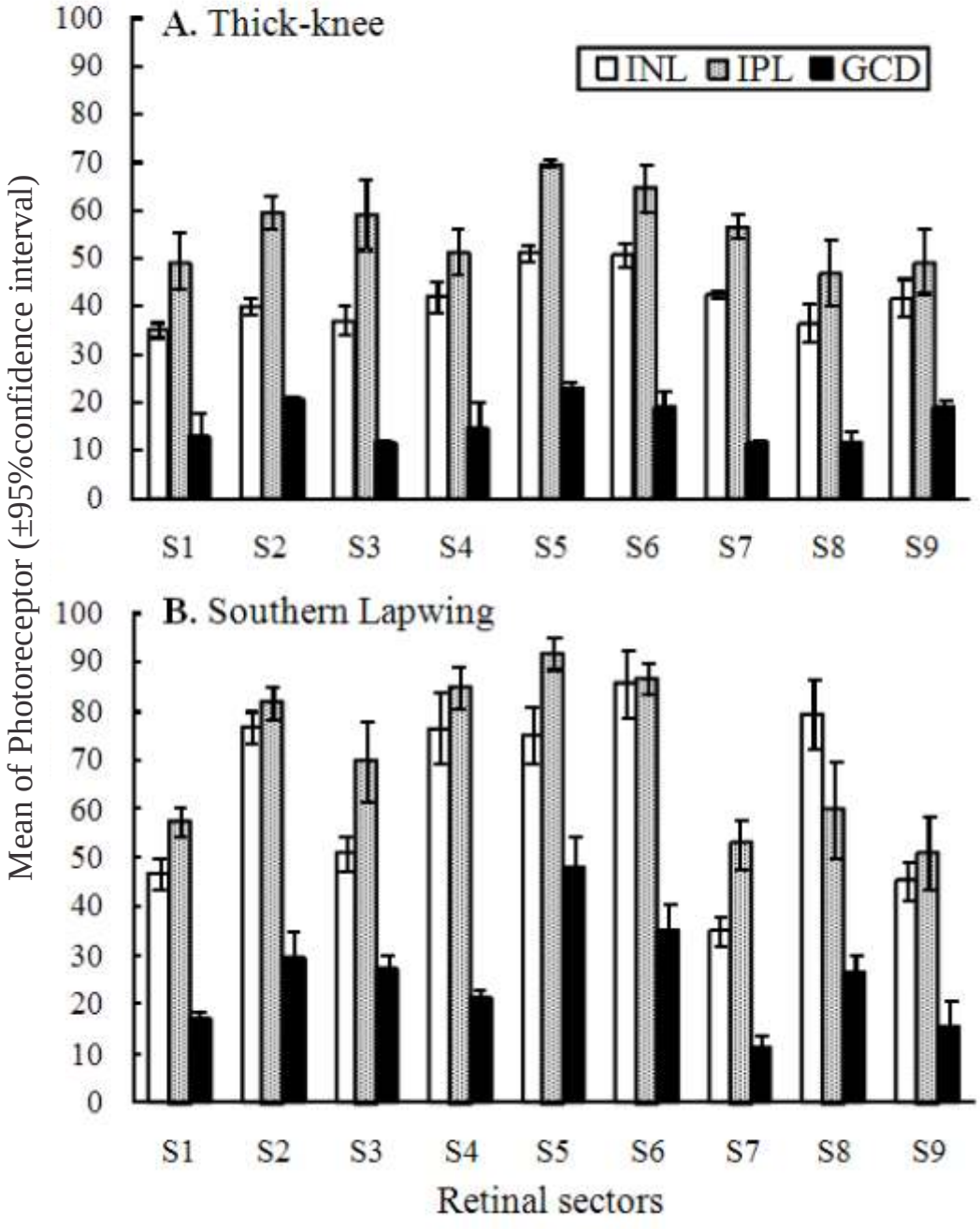

Figure 6. Mean thickness (µm) of retinal layers and mean ganglion cell density per 238 µm in each of the nine retinal sectors of the species studied. Columns represent the means (±95% CI).

Table 1. Eyes (mm) and mean rod and cone measurements (µm) of the species studied, and comparisons with other species.

Spp.	n	Feeding habit	Axial length (mm)	Equatorial diameter (mm)	AL/ED	Dilated pupil diameter	Rod:Cone	Rod outer segment length	Rod outer segment diameter	Cone outer segment length	Cone outer segment diameter	Ganglion cell numbers
Burhinus bistriatus[1]	4	Crepuscular and nocturnal	21.0±0.9	25.8±0.3	0.81	9.0	3.0 : 1	64.7±2.6	3.7±0.4	16.5±1.9	1.00±0.03	24.6±1.3
Vanellus chilensis[1]	4	Mainly diurnal; crepuscular	13.0±0.9	18.5±0,1	0.70	7.0	0.7 : 1	44.3±1.7	4.9±0.07	20.0±1.9	2.98±0.04	46.9±2.7
Charadrius wilsonia[2]	4	Diurnal and nocturnal	11.2±0.3	13.1±0.6	0.86	5.2±0.3	1.3 : 1	47.2±4.2	3.8±0.2	17.6±1.7	1.3±0.1	45.2±5.9
Limnodromus griseus[2]	4	Diurnal and nocturnal	7.2±0.1	9.5±0.1	0.76	4.0±0.0	1.0 : 1	33.5±2.0	4,1±1.2	15.5±0.9	1.4±0.1	34.1,±2.6
Himantopus himantopus[2]	10	Diurnal and nocturnal	13.2±0.5	15.4±0.3	0.86	6.0±0.4	1.1 : 1	41.2±1.3	4.1±0.1	18.5±1.0	1.3±0.1	33.6±2.6
Catoptrophorus semipalmatus[2]	7	Diurnal and nocturnal	12.0±0.9	14.5±0.4	0.83	4.2±0.2	0.7 : 1	35.6±4.07	3.9±0.1	17.5±2.1	1.3±0.1	38.3±2.2
Scolopax minor[2]	6	Diurnal and nocturnal	10.6±0.2	15.0±0.3	0.71	7.3±0.8	1.2 : 1	48.4±12.3	3.9±0.1	21.9±3.8	2.0±0.3	30.3±0.9
Rynchops niger[3]	4	Crepuscular and nocturnal	2.9±0.8	15.5±0.6	0.83	7.2±0.5	5.0 : 1	25.2±5.9	1.9±1.1	8.2±0.9	1.3±0.1	13.5±4.6

[1]This study; [2]Rojas *et al.* 1999a; [3]Rojas *et al.* 1997.

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