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Development of the Superaltricial Monk Parakeet (Aves, Psittaciformes): Embryo Staging, Growth, and Heterochronies

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ABSTRACT

Knowledge about the embryonic stages of birds is important in answering many questions about development and evolution. We give the first description of 41 embryological stages of the monk parakeet (*Myiopsitta monachus*) on the basis of external morphology and comparison with the chicken. We also provide measurements of some external morphological characters (i.e. body mass, crown-rump, beak, forelimb, and third toe lengths) and perform comparisons with other precocial and altricial birds with the aim of identifying heterochronous developmental features. The following differences in the development of characters in the monk parakeet when compared with other birds were found: (1) delay of the feathers primordia, (2) wing buds initially greater than leg buds, (3) forelimbs and hindlimbs with similar relative size, (4) retroversion of the toe IV, (5) ventral curvature of the upper jaw, (6) positive regressions between stages and beak length with acceleration and higher values and III toe lengths with deceleration and lower values in the monk parakeet compared to the chicken. The growth pattern of the monk paraket *Myiopsitta monachus* could be influenced by some heterochronic processes like post-displacement, acceleration and/or deceleration. Results of this research allow the standard identification of stages in different species of parrots, recognize similarities and differences between precocial (the chicken) and altricial species (*Myiopsitta*), and provide planning data for future studies. Anat Rec, 298:1836–1847, 2015. © 2015 Wiley Periodicals, Inc.

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Knowledge about the embryonic stages of birds is important in answering many questions in development, evolution, and conservation. Biologists have long sought to understand the relationship between ontogeny and phylogeny, particularly after the Haeckel's and von Baeŕs drawings of embryos in the late nineteenth century (Gould, 1977; Hopwood, 2007). The interest in avian development had a significant relaunching a decade ago with the Evo-Devo that aims to understand the mechanisms governing the evolution of development. Heterochronies or evolutionary changes in the timing and/or rates of processes underlying the ontogenetic formation of morphological traits (Reilly et al., 1997) has been extensively studied. Most of the literature is based on Gould (1977) and Alberch et al. (1979) approaches, but the models proposed by the authors led to ambiguity in

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the application of certain concepts (Reilly et al., 1997). In his classic review, Reilly et al. (1997) proposed six processes of heterochronic change that result from shifts in rate, offset, or onset of the developmental trajectories. They include: acceleration and deceleration (in replacement of neoteny) resulting from an increase or decrease of growth rate; hypermorphosis and hypomorphosis (in replacement of enanism) as a result of an extended or reduced offset time; and predisplacement and postdisplacement when the onset time of a structure begins before or after respectively. These processes can produce truncated (paedomorphosis), extended (peramorphosis) or the same (isomorphosis) traits when comparing two ontogenetic trajectories. The study of comparative development gives the empirical basis for advances on this matter.

Developmental biology is unimaginable in contemporary science without standard definition of normal stages (Hopwood, 2007). Widely used as model is the chick embryós development, extensively studied by many researchers and discretized in 46 chronological stages by Hamburger and Hamilton (1951). Since then, the normal stages of the embryo development of *Gallus gallus domesticus* have been applied as a model to solve several questions such as migration of germ cells, cellular and molecular interactions during development, and production of transgenic birds and chimeras (Ainsworth et al., 2010; Le Douarin and Dieterlen-Lièvre, 2013). Nevertheless, knowledge about prenatal development in birds grew very slowly. Complete or partial embryonic development is known of few precocial or semiprecocial birds, particularly poultry and/or species of commercial importance like chickens, quail and other Phasianidae, and ducks (e.g. Fant, 1957; Rempel and Eastlick, 1957; Koecke, 1958; Mun and Kosin, 1960; Phillips and Williams, 1964; Hendrickx and Hanzlik, 1965; Kaltofen, 1971; Dupuy et al., 2002; Ainsworth et al., 2010; Ramteke et al., 2013).

A few works have described all or some of the developmental stages of wild birds like Society finch *Lonchura striata* (Yamasaki and Tonosaki, 1988), Brandt cormorant *Phalacrocorax penicillatus* (Price, 1938), Barn owl *Tyto alba* (Köppl et al., 2005), emu *Dromaius novaehollandiae* (Nagai et al., 2011), lapwing *Vanellus cristatus* (Grosser and Tandler, 1909), Adélie penguin *Pygoscelis adeliae* (Herbert, 1967), black stilt *Himantopus novaezelandiae* (Browne, 2006), and rock pigeon *Columba livia* (Olea and Sandoval, 2012).

Works regarding embryological development of Psittaciformes are scarce, incomplete, and restricted to Australian and African species. Braun (1879), Keibel (1901), and Abraham (1901) were the first to study embryos of the Psittacidae budgerigar *Melopsittacus undulatus*. Also Mebes (1984) made observations on the embryogenesis of the Rosy-faced lovebird *Agapornis roseicollis*. Tokita (2004) was the only researcher to perform a description of the last normal developmental stages (from stage 17 onwards) of a member of the Cacatuidae family, the cockatiel *Nymphicus hollandicus*, although brief, superficial and focused on the morphogenesis of jaw muscles.

The monk parakeet (*Myiopsitta monachus*, Boddaert 1783) is one of the few Neotropical species whose capture has no restrictions because its pest status (Canavelli et al. 2013), providing benefits for the abundant availability of specimens. Also is an interesting parrot to study because of all Psittaciformes, it is the only one that builds a stick nest instead of using a hole in a tree and breeds in a single large nest with separate entrances for each couple (Collar, 1997 and bibliography cited therein). Monk parakeet eggs are small, white, and measure 3 cm in length approximately. Clutch size is 7 on average (one deposited every two days), and the incubation period is 24 days. The superaltricial chicks hatch with eyes closed, nearly without feathers and stay in the nest until 40 days after hatching (Navarro and Bucher, 1992; Navarro et al., 1995; Salvador and Aramburú, 1995).

To the best of our knowledge, this is the first thorough investigation dedicated to the development of parrots giving the first description of embryological development of a Neotropical parrot, the monk parakeet *Myiopsitta monachus*, and the first complete staging of any Psittaciformes embryo. We provide measurements of some external morphological characters and performs comparisons with other precocial (e.g. chicken) and altricial (e.g. cockatiel) birds.

Our objectives were to identify heterochronus developmental features, to provide normative information that would enable standard identification of stages in different species of parrots, to recognize similarities and differences between the chicken (precocial) and *Myiopsitta* (altricial) species, and to provide planning data for future studies.

MATERIALS AND METHODS

A total of 200 specimens of monk parakeet *Myiopsitta monachus* were used in the development staging series. Eggs were obtained from nests in Dean Funes locality, Córdoba province (Argentina) during October and November of the breeding periods of 2012 and 2013. The embryos were removed with forceps from the extraembryonic membranes. Each embryo was weighed with a digital scale (0.001 g precision) and fixed by immersion in a 4% neutral buffered formaldehyde solution. The external morphology of early stages embryos was studied under a stereomicroscope Leica S6D. To enhance structure visibility (e.g. somites, pharyngeal arches, limb buds, eyelids, feather germs) a 0.5% neutral red in PBS (phosphate buffered saline solution) staining solution was used (technique based on that used by Ainsworth et al., 2010). Due to the great height of nests and their composition of interlaced sticks, access to eggs was difficult and it was not possible to keep track of incubation time in the field, so stage designation was made only on the basis of external morphology and comparison with Hamburger and Hamilton (1951) stages.

The assignment of stages was performed based on a set of "events" taking into account some morphological features. These are morphological states of one or more embryological structures (arbitrarily selected by Hamburger and Hamilton, 1951) that define a stage and distinguish a previous stage from the next (Bininda-Emonds et al., 2002). When the size and degree of embryo development allowed it, crown-rump length, beak length (from the base to the tip), forelimb autopodium length, and third toe length including claw were measured using a digital calliper to the nearest 0.01 mm. All measurements were log10-transformed and descriptive statistics, growth curves, correlation and linear regression analysis were performed using the Past 3.02a software (Hammer et al., 2001). The results were compared with those available for other species in the literature (i.e. *Nymphicus*, Tokita, 2004; and *Gallus*, Hamburger and Hamilton, 1951). Because this study presents data from relatively few individuals per stage, non-parametric statistics were applied. Finally, photos were taken with a Nikon D-40 digital camera (macro 128 mm).

RESULTS

Developmental Stages Description

Stage 1. After laying. The peripheral area opaca and the central area pellucida are evident.

Stage 2. The primitive streak has triangular shape. It is observed in the outer margin of the area pellucida and it extends toward its center (Fig. 1A).

Stage 4. The area pellucida becomes pear-shaped. The primitive streak reaches its maximum length. The primitive groove, primitive pit and Hensen's node are observed (Fig. 1B).

Stage 8. The neural folds meet at the anterior region. Four pairs of somites and blood islands are present.

Stage 9. Primary optic vesicles begin to form. Six pairs of somites are distinguishable (Fig. 1C).

Stage 11. All primary brain vesicles are evident: telencephalon, diencephalon, mesencephalon, metencephalon and myelencephalon. Slight cranial flexion. The anterior neuropore begins to close. From a ventral view the triangular heart with a slight bent to right can be observed. Thirteen pairs of somites are distinguishable (Fig. 1D).

Stage 15. Cranial and cervical flexures. Hindbrain nearby the heart, oriented ventrally and forming a 90° angle with the midbrain. Pineal gland visible. Three pharyngeal arches evident. Otic and optic vesicles present. Heart with tubular torsion. Tail bud begins to form. Somites hard to count. No wing or leg buds are evident (Fig. 2A).

Stage 19. Defined body with head, lateral and tail folds complete. Head with brain parts delimited. Hindbrain contacts the heart. Pineal gland present in the diencephalon. Eyes without pigmentation. Otic vesicles dorsal to the pharyngeal arches. Nasal pits located in the ventro-lateral part of the head. Four pharyngeal arches evident, the second arch is the largest. The mandibular process is longer than the maxillary process. Limb buds present and greater in width than length. Forelimbs larger than hindlimbs. Somites extends to tail that begins to curve. Amnion closed and small allantois present.

Stage 20. Head and tail folds converge in the umbilical area. Eye pigmentation faint. Pharyngeal arches conspicuous. Limb buds greater in width than length. Forelimbs larger than hindlimbs. Allantois with vesicular shape and similar size that the telencephalon (Fig. 2B).

Stages 23. Telencephalic vesicles are distinguished. Eye pigmentation evident. Maxillary and mandibular processes similar in length. Limb buds equal in width and length. Forelimbs larger than hindlimbs (Fig. 2C).

Stage 24. Maxillary process anterior to lens and longer than the mandibular process. Distal tip of the mandibular process at the mid-line of the eye. Limbs greater in length than width (Fig. 2D).

Stage 25. Presence of protuberances delimiting the external auditory opening and forming a collar dorsally to the pharyngeal arches. Optic fissure evident. Limbs with elbow and knee distinct and with rounded distal digital plate (Fig. 3A).

Stage 27. The region of the beak can be identified. Maxillary and mandibular processes anterior to the eye. External nares evident. Elongated limbs, with enlargement of the distal plate. Urogenital papilla evident (Fig. 3B).

Stages 29. Eyelids begin to grow. Beak defined with egg tooth. The collar disappeared. Elongated neck. Distinguishable auditory meatus. Limbs with three segments delimited (stylopod, zeugopod, and autopod). All fingers distinct by grooves and with interdigital webs.

Stages 31. Some scleral papillae are distinguishable in the caudo-ventral margin of the eye. Fingers elongated with thin and concave interdigital webs. First feather primordia in two rows in dorsal region, limbs base and tail (Fig. 3C).

Stage 34. Eyelids start to grow and feather primordia are present around them. The nictitating membrane begins to grow in the anterior region of the eye. Up to 14 scleral papillae in eye. Beak elongated and more defined. Maxillary process anterior to the telencephalon and longer than mandibular process, the latter with serrated edges at the anterior end. Egg tooth cornified. Elongated limbs with interdigital webs reduced. In the forelimbs, the alula is differentiated. The digit II of the forelimb is elongated. Feathers primordia on the cervical region, the ventral edge of the forelimbs, base of the humerus, dorsal and ventral region of the trunk (except central rows), hindlimbs, and tail (Fig. 3D).

Stage 35. Brain projections disappear. The nictitating membrane approaches to the sclerotic papillae. Serrated edges at the anterior end of both maxillary and mandibular processes. Interdigital webs absent. In the forelimbs, the digit II is more elongated than in previous stage. In the hindlimbs, the toe phalanges are evident, toe IV starts to rotate laterally and the hallux medially. The urogenital papilla is globular. The old feather primordia have grown in length. Feathers germs also on frontals and nasals, eyelids, auditory meatus, cheeks, and neck (Fig. 4A).

Stages 36. Ellipsoidal and translucent eyelids. Cere surrounding external nares. Toe rotation complete. Claws of hindlimbs toes slightly curved. Feather primordia lengthened in the dorsal region and pygostyle (Fig. 4B).

Stages 38. Lower eyelid covers half of the eye. Egg tooth and surrounding areas cornified. Primordia of scales are evident in hindlimbs (Fig. 4C).

Stage 40. Eyelids are translucent and close the eyes. Beak almost fully cornified and with fleshy commissures. Cornified and curved claws are evident. Feathers yellow colored and lengthened, especially in the dorsal region, hindlimbs, and pygostyle.

Stage 40+ (hatchling). Eyelids not translucent. Beak fully cornified. Ventrally curved maxilla with lateral tomial teeth. Curved toes with fully cornified claws. Cloaca defined. Swollen crop and abdomen (Fig. 4D).

As a result of our sampling, there is a restricted availability of a set of embryos of continuous stages.

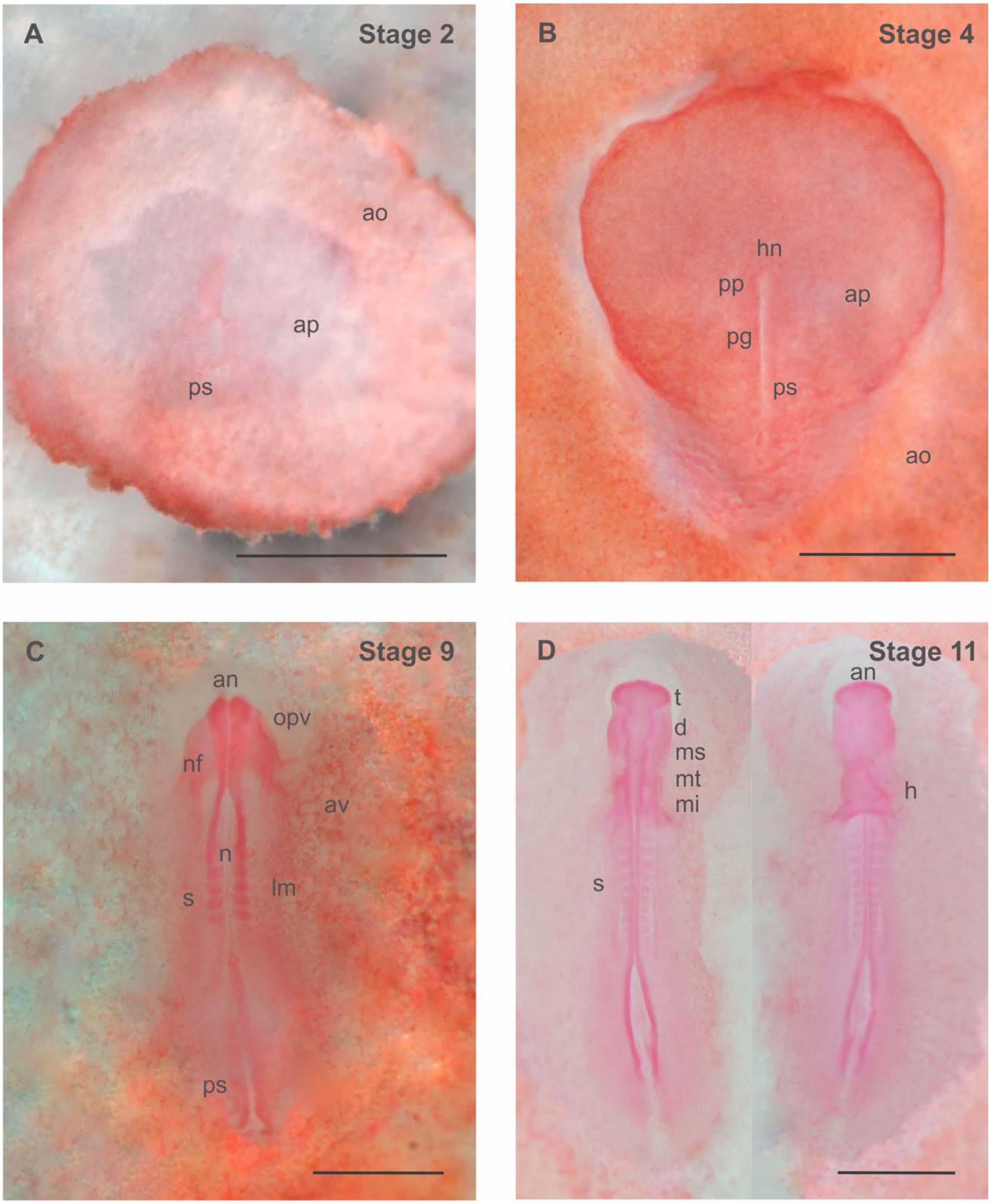

Fig. 1. Developmental stages of *Myiopsitta monachus*. **A**: stage 2; **B**: stage 4; **C**: stage 9; and **D**: stage 11 in dorsal (left) and ventral (right) views. Abbreviations: an, anterior neuropore; ao, area opaca; ap, area pellucida; av, area vasculosa; d, diencephalon; h, heart; hn, Henseńs node; lm, lateral plate mesoderm; mi, mielencephalon; ms, mesencephalon; mt, metencephalon, n, notochord; nf, neural folds; opv, optic vesicles; pg, primitive groove; pp, primitive pit; ps, primitive streak; s, somites; t, telencephalon. Scale bar: 1 mm.

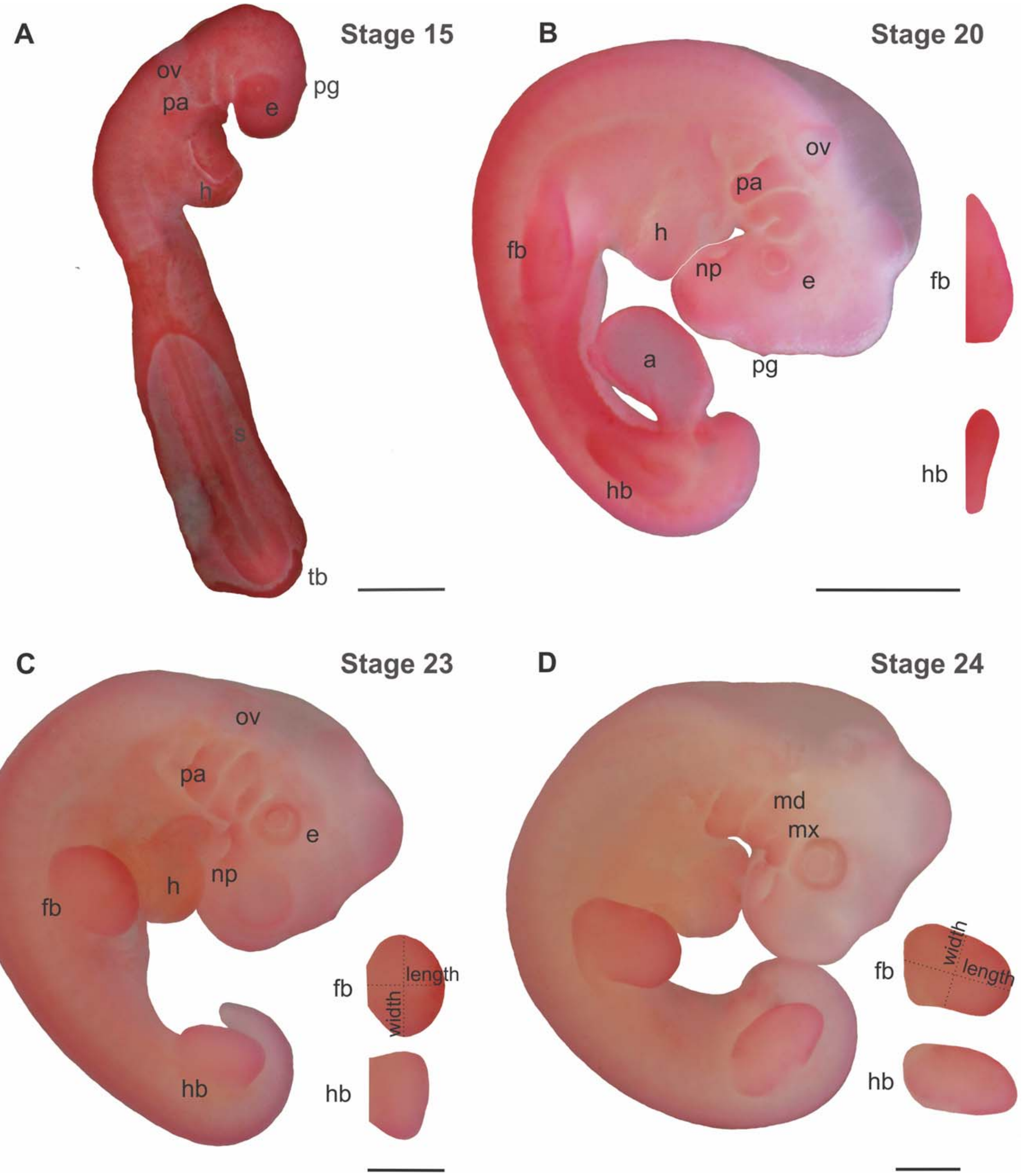

Fig. 2. Developmental stages of *Myiopsitta monachus*. **A**: stage 15; **B**: stage 20; **C**: stage 23; and **D**: stage 24 in lateral views. Abbreviations: a, alantoids; an, anterior neuropore; e, eye; fb, forelimb bud; h, heart; hb, hindlimb bud; md, mandibular process; mx, maxillary process; np, nasal pit; ov, otic vesicle; pa, pharyngeal arches; pg, pineal gland; s, somites; tb, tail bud. Scale bar: 1 mm. Detail of limbs not in scale.

Morphological Measurements and Growth

Quantitative measurements were taken for monk parakeet embryos in order to provide developmental characterization as a baseline. Average embryo body masses, crown-rump length (CRL), beak length, forelimb autopodium length, and third toe length including claw are presented in Table 1. Body mass and CRL were

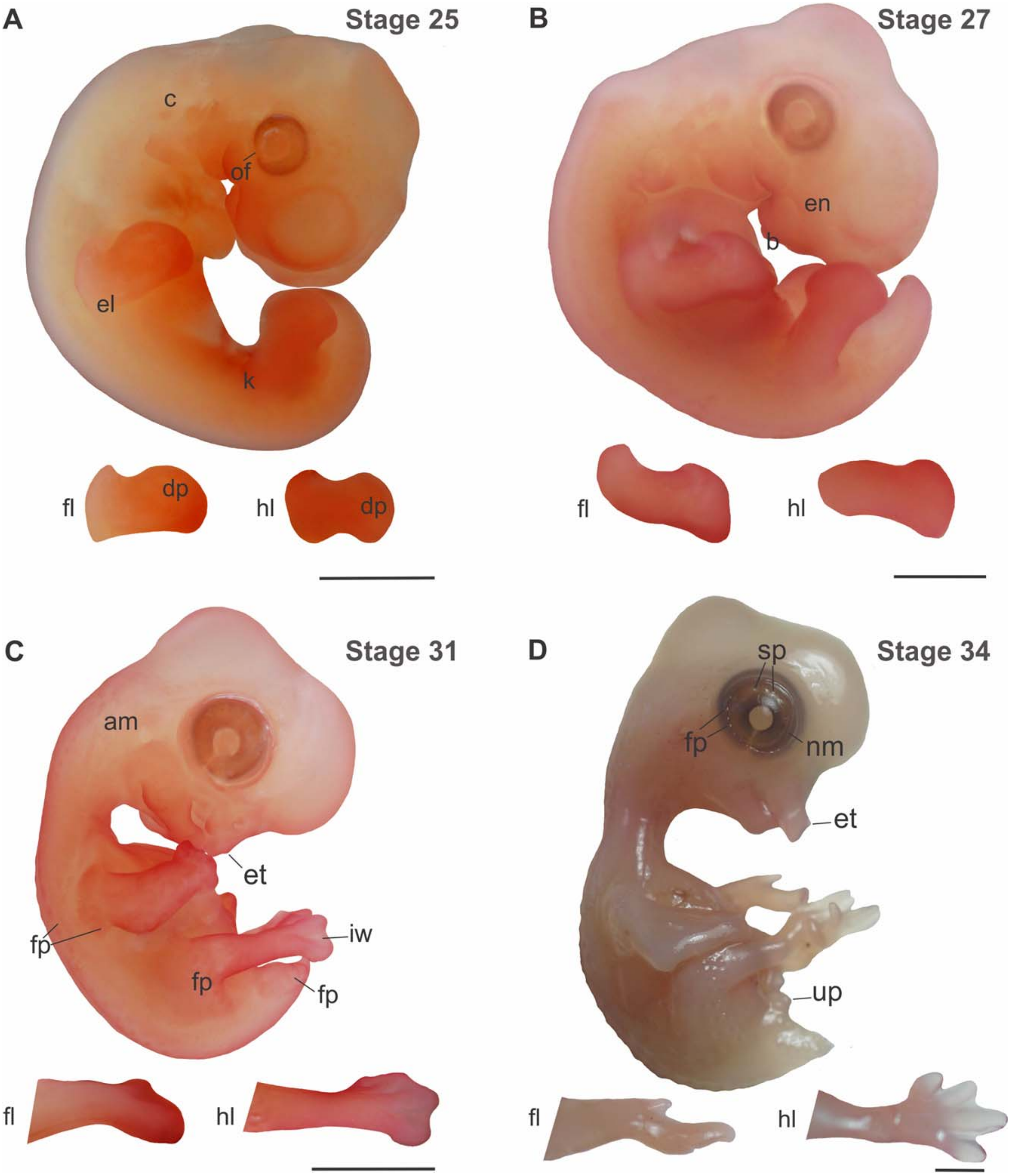

Fig. 3. Developmental stages of *Myiopsitta monachus*. **A**: stages 25; **B**: stage 27; **C**: stage 31; and **D**: stage 34 in lateral views. Abbreviations: am, auditory meatus, b, beak; c, collar; dp, digital plate; el, elbow; en, external nares; et, egg tooth; fl, forelimb; fp, feathers primordia; hl, hindlimb; iw, interdigital webs; k, knee; nm, nictitating membrane; np, nasal pit; of, optic fissure; up, urogenital papilla. Scale bar: 2 mm. Detail of limbs not in scale.

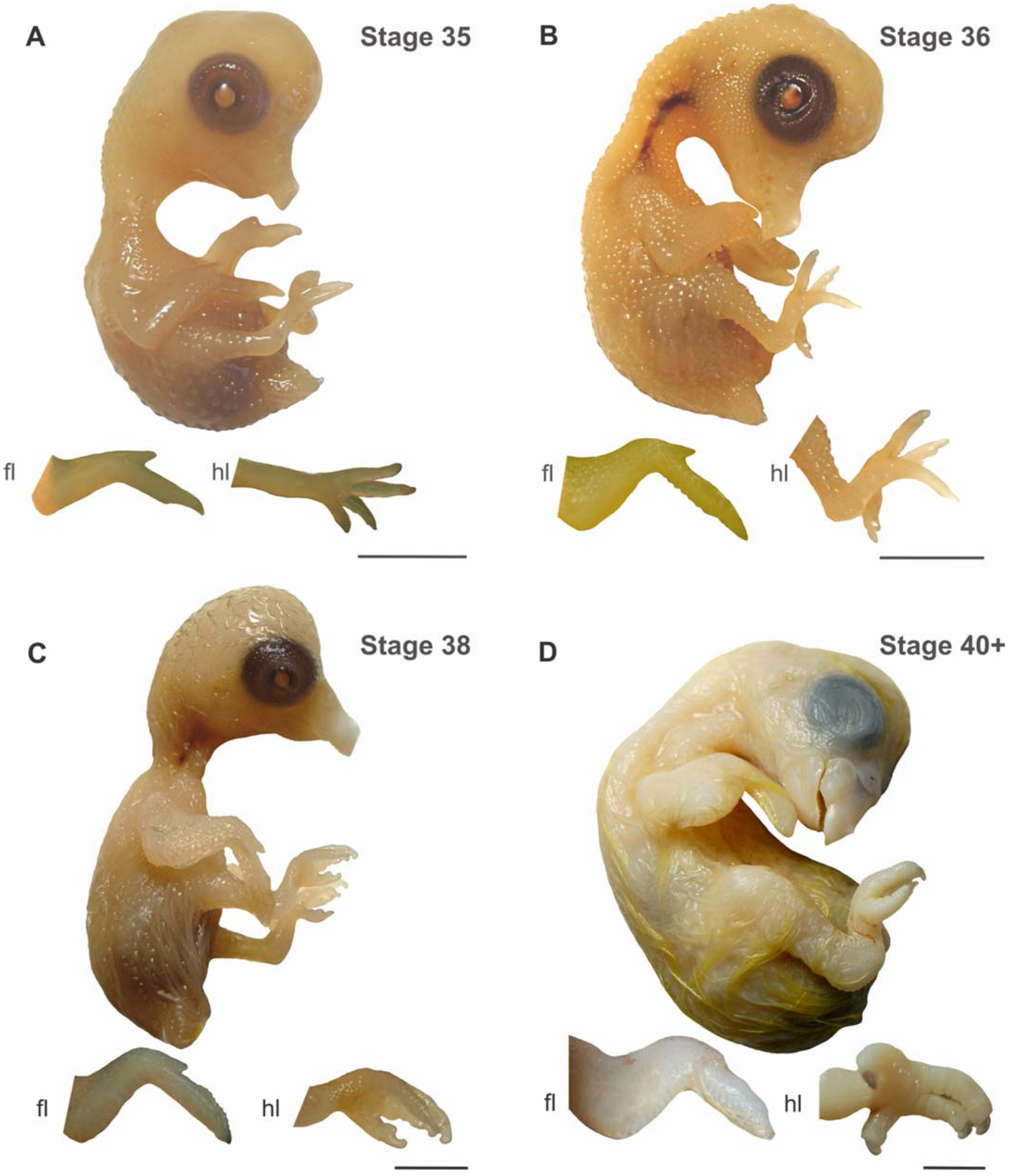

Fig. 4. Developmental stages of *Myiopsitta monachus*. **A**: stage 35; **B**: stage 36; **C**:, stage 38; and **D**: stage 40+ in lateral views. Abbreviations: fl, forelimb; hl, hindlimb. Scale bar: 5 mm. Detail of limbs not in scale.

measured from stage 24 while the other measurements from stage 34 onwards.

Correlation analysis showed a positive relation between body mass and CRL ($r^2 = 0.96$, $P < 0.05$; Fig. 5A). The growth curves of external morphological characters (beak length, forelimb and third toe length) as a function of embryonic mass are given in Fig. 5B–D. Besides shape change, the growth of the beak showed an increase in size faster in the early stages (34 to 36) with a slow decrease in the growth rate towards the end of the embryonic stages. Third toe length followed a similar pattern while forelimb length showed a faster increase in the early stages (34 to 36), and then remained relatively constant in stages 36 to 40 and increased again in the final stages.

DISCUSSION

How the Development of the Monk Parakeet Fits in the Chick Embryo Model?

Hamburger and Hamilton (1951, HH) described a series of 46 stages of the *Gallus gallus domesticus* chick development, a species model widely used in the embryological studies of birds (Bellairs and Osmond, 2005; Davey and Tickle, 2007; Vergara and Canto-Soler, 2012).

TABLE 1. Developmental stages of *Myiopsitta monachus* embryos and its average measurements (with SE, standard error; df: n – 1) from stage 24 onward

Stage	N	M	CRL	Beak	FL	HL dIII
24	3	0.103 ± 0.049	5.147 ± 0.689	–	–	–
25	14	0.131 ± 0.028	7.041 ± 1.827	–	–	–
27	16	0.234 ± 0.033	9.718 ± 2.106	–	–	–
29–30	7	0.344 ± 0.044	10.236 ± 1.743	–	–	–
31–33	16	0.412 ± 0.052	13.007 ± 2.282	–	–	–
35	11	0.780 ± 0.080	18.008 ± 1.024	3.458 ± 0.285	4.736 ± 0.531	2.363 ± 0.221
36–37	28	1.095 ± 0.124	20.888 ± 1.928	4.420 ± 0.502	5.483 ± 0.620	3.159 ± 0.396
38–39	24	1.561 ± 0.281	23.950 ± 3.502	5.160 ± 0.354	5.838 ± 0.388	3.773 ± 0.375
40	28	2.509 ± 0.431	29.079 ± 4.021	6.022 ± 0.543	5.965 ± 0.600	4.368 ± 0.479
41	13	3.810 ± 0.385	30.033 ± 3.962	6.808 ± 0.328	7.117 ± 0.620	4.893 ± 0.339

Abbreviations: n, number of specimens; *M*, body mass (in grams); CRL, crown-rump length (in mm); Beak, beak length (in mm); FL, forelimb autopodium length (in mm); and HL dIII, third toe length including claw (in mm).

Developmental stages are an appropriate tool for the comparison of development within Aves since they standardize homologous structural events independently of time and body size, and changes from the normal stages could indicate heterochronic events (Starck, 1993; Rice, 1997). This approach is useful to compare not only embryo growth of the same avian species but also in an interspecific comparative frame. However, HH characterizes their last stages only based on the length of beak and third toe and, to some extent, this makes it inapplicable in a comparative context. Ricklefs and Starck (1998) in a shorter model consider that the stage 40 is the sum of 40-44 HH stages and stages 41 and 42 are characterized by behaviour of the embryo, pipping, and hatching respectively. Our description follows this second scheme (40+ stages).

Comparison Between Development of the Monk Parakeet and Other Aves

The embryonic development of birds in general is morphologically conserved, although variable in growth rates (Nagai et al., 2011). We found differences in development of characters in the monk parakeet when compared with those of the chicken and other birds, which evidence some heterochronic events mainly in the latest stages.

Parrots and galliforms were long thought to be distant relatives. In contrast to parrots, galliforms are precocial birds whose offspring follow their parents and feed on their own (Ricklefs and Starck, 1998). Altricial offspring remain relatively helpless and dependent on their parents after hatchling. This is in line with the delayed maturation of the legs, plumage, vision, and also of the brain growth as indicated by Charvet and Striedter (2008) and Charvet et al. (2011). In most phylogentic schemes (e.g. Jarvis et al., 2014), Galliformes are considered basal while parrots are derived; also, precociality is the ancestral condition regarding altriciality (Starck, 1993). In this context, here direction of changes is determined in *Myiopsitta* compared with *Gallus*. The appearance of the feathers primordia occurs later in the monk parakeet (stage 31) and in the cockatiel (stage 32; Tokita, 2004) when compared with the chicken (stage 30; Hamburger and Hamilton, 1951). As consequence, the newborn chick has virtually no external feathers, a feature shared with other altricial species. This difference in the time of appearance of feathers primordia could indicate a post-displacement and afterwards acceleration processes in *Myiopsitta* because isomorphic adults are produced (complete plumage in both species). Similar processes might occur in the semialtricial Barn owl that hatches covered with plumage and which feathers germs appear also in the stage 32 of development (Köppl et al., 2005).

Wing buds are initially greater than leg buds in the monk parakeet, similar condition to that described for the cockatiel (Tokita, 2004) and different from the chicken (Hamburger and Hamilton, 1951). Also, we found that forelimbs and hindlimbs have similar relative size in the monk parakeet, contrary to the chicken that shows a greater size of the hindlimbs (Hamburger and Hamilton, 1951). This differential development degree of the limbs could be due to changes in the rate of development in *Myiopsitta* when compared with *Gallus*: initially there is an acceleration of the wing buds and a deceleration of the leg buds followed by a deceleration of hindlimbs. These features are associated with different locomotor habits between the flying monk parakeet and the more terrestrial chicken, a fact that is evident from the moment of hatch. Differences in the growth rate of limbs have been demonstrated in other species, for example, the precocial and cursorial ostrich and emu which have a noticeable reduced growth rate in the forelimbs (Blom and Lilja, 2005; Nagai et al., 2011). As well, in the altricial and flying fieldfare the forelimbs are significantly larger than the hindlimbs (Blom and Lilja, 2005).

During the stages 35 and following, substantial changes occur in the foot. Since the chicken have anisodactyl foot and the monk parakeet zygodatil one, it is not surprising that the major differences between both species happen at these last stages (see below). The peculiar ventral curvature of the upper jaw occurs in the later stages of the intra egg development at stage 40+ in the monk parakeet. Therefore, the chicks have their characteristic beak at hatch but begin to feed themselves 20 days after that. Images available of the cockatiel (Tokita, 2004) allow inferring that the same happens in this species. Of course, this does not occur in the chicken embryo.

In sum, prominent differences exist between precocial and altricial embryos at least in the later stages. In addition, certain characters present in the same stage, may have significant qualitative differences that are particularly manifest after hatchling. For example, the

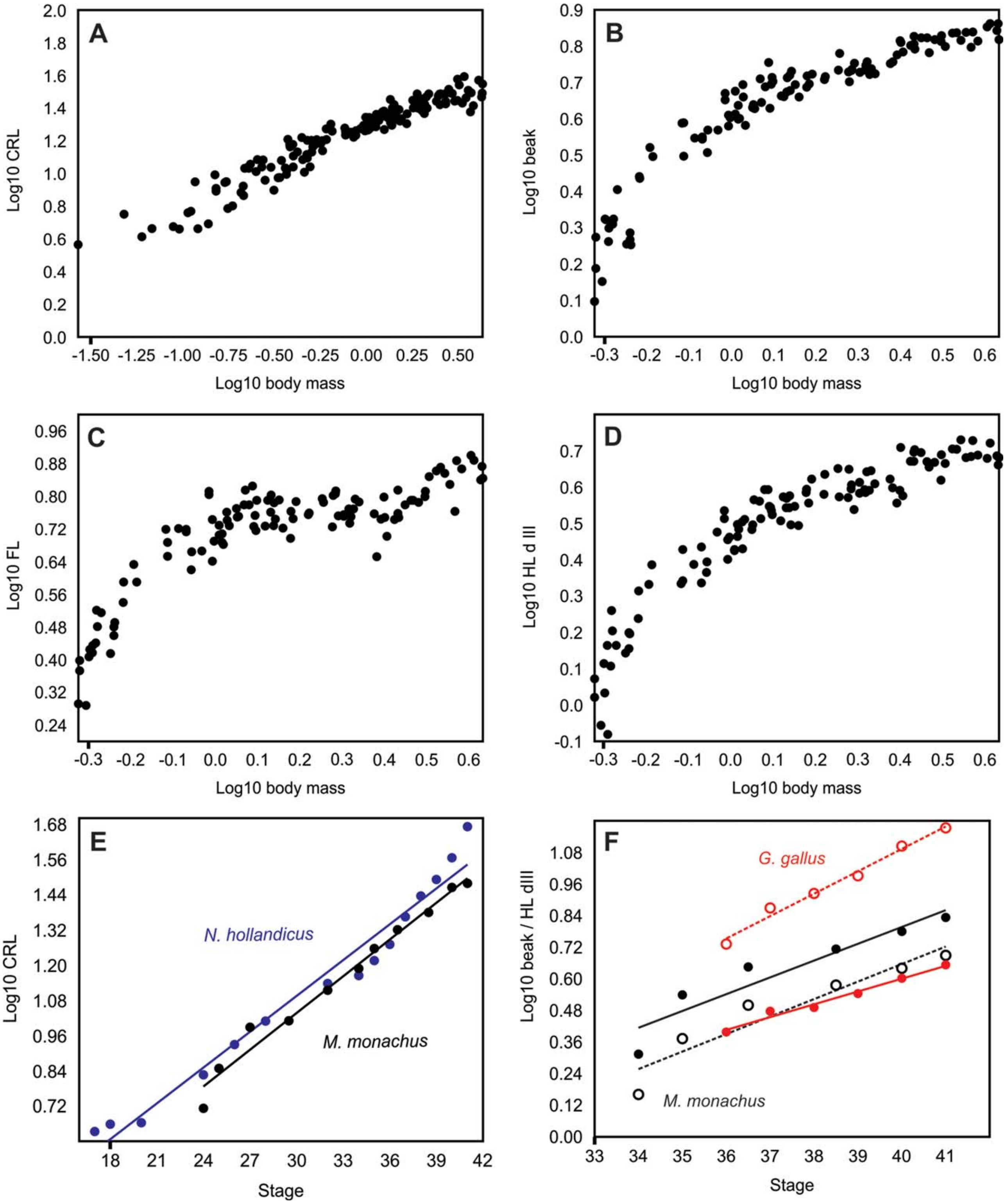

Fig. 5. Scatterplot of $\log_{10}$ measurements versus $\log_{10}$ body mass in *Myiopsitta monachus* (**A–D**). Scatterplot of correlation of $\log_{10}$ CRL versus stages in *Myiopsitta monachus* compared with *Nymphicus hollandicus* (**E**), and linear regressions of beak length and III toe length (dotted line and empty circles) compared with *Gallus gallus* domesticus (**F**).

density of feathers (high in the chicken and low in parrots), and the ability to open the eyes (the chickens are born with their eyes open while parrots with eyes closed).

Morphological Measurements and Growth Comparisons

Quantitative measurements of external morphological characters for the monk parakeet embryos proved to be useful in developmental characterization and to make comparisons with other birds.

A positive correlation between stages and CRL was found in both the monk parakeet ($r^2 = 0.98$, $P < 0.05$) and the cockatiel ($r^2 = 0.98$, $P < 0.05$). In the latter, CRL values are higher throughout the whole development (Fig. 5E), probably due to its larger size (adult *N. hollandicus* 33 cm; Rowley, 1997; and adult *M. monachus* 28–29 cm; Collar, 1997). Also, cockatiels have a growth spurt of CRL at later stages of development whereas the monk parakeet could have a decrease of growth rate (deceleration).

In comparison with the available data on the precocial chicken (Hamburger and Hamilton, 1951), the positive regressions between stages and beak length showed acceleration (higher slope) and higher values for the monk parakeet (Fig. 5F; *G. gallus*, $r^2 = 0.98$, $p<0.05$, slope 0.04; *M. monachus*, $r^2 = 0.88$, $P < 0.05$, slope 0.06), which results in larger beaks with higher growing rates. Conversely, positive regressions between stages and III toes lengths showed deceleration (lower slope) and lower values for the monk parakeet in comparison with the chicken, which results in shorter III toes with lower growing rates (Fig. 5F, dotted lines; *G. gallus*, $r^2 = 0.98$, $P < 0.05$, slope 0.08; *M. monachus*, $r^2 = 0.89$, $P < 0.05$, slope 0.06). Even though the correlation coefficients for the chicken are higher than they are for the monk parakeets, “r” values are close to one in both species and there are no significant differences between them.

Acquisition of Zygodactil Arrangement of Toes

Study of digits position is interesting for several reasons: implies a deep transformation or repositioning, because the type of foot is usually employed for establishing relationships within Aves, and because one of the ongoing discussions in the field of ornithology concerns homologies between fingers. A zygodactyl or yoke-toed foot (digits II and III directed forwards, digits I and IV pointing backwards) is a specialization of the avian leg identified with perching, climbing or manipulation habits (Carril et al., 2014 and bibliography cited therein). It is considered a modification of the anisodactyl foot (digits II, III and IV oriented forward) that evolved independently at least in two other clades other than Psittaciformes (Cuculidae and Piciformes; Mayr, 2014). In the monk parakeet, the retroversion of digit IV starts during the stage 35 (Fig. 4A) and is complete at stage 36. These observations are completely consistent with that seen in the budgerigar (Botelho et al., 2014). To our knowledge, there is no more information in the literature about the lateral rotation of digit IV in any other Psittaciformes. Tokita (2004) makes no reference to this aspect about the cockatiel development. Finger IV rotation occurs after the reduction (by apoptosis) of the interdigital webs that binds all fingers from stage 29 to 34 in both parrots: monk parakeet (this paper) and the budgerigar (Botelho et al., 2014). Comparing with other birds, between stages 29 and 35 the deeper changes in the foot occur. In the chicken, webs between digits and toes disappear during stage 35 and they become separated (Sanz-Ezquerro and Tickle, 2003; Bellairs and Osmond, 2005). During the stage 29 of the Barn owl development, the four toes have webs; toes with slightly concave webbing in between are visible during stage 32 and have nearly disappeared during stage 35 (Köppl et al., 2005).

Another important process during digit morphogenesis is the reduction (in parrots) or not (in the chicks’ embryo) of the *musculus extensor brevis digiti IV* during the stage 35 (Botelho et al., 2014). These authors states that the decrease may be related to the retroversion of the 4th digit in the budgerigar. The *musculus extensor brevis digiti IV* is also completely absent in the monk parakeet (Carril et al., 2014).

The digit formation and growth can provide important information about evolutionary morphological diversity. Particularly, the development of the zygodactyl foot could supplement morphological systematic and evolutionary studies of Psittaciformes, relationships within Telluraves (the clade including most arboreal birds in the sense of Yuri et al., 2013) and other Neornithes.

CONCLUSIONS

Comparative embriology is the empirical basis for discover how developmental processes have evolved and is the motor of contemporary field of Evo-Devo. At the dawn of the studies on avian development, chick embryo was used as a useful model and has become one of the most versatile systems in developmental biology (Stern, 2004), even in the incredibly fast-growing molecular science. Considering that birds are the most diverse continental vertebrates, knowledge about their development is scarce. Our work partially fills that gap. Most developmental stages proposed Hamburger and Hamilton (1951) for the chicken can be recognized in the development of the monk parakeet and the main differences between species were found in the later stages. Standardizing the stages of development is an important tool in comparative studies of embryology. Our study is the first dedicated to the development of a Neotropical parrot and is among the few dedicated to Psittaciformes in general. Most of the morphological variation within groups is a consequence of changes in developmental timing and rates (heterochrony). The growth pattern of the monk paraket *Myiopsitta monachus* may be the product of a combination of heterochronic processes like post-displacement, acceleration and/or deceleration.

Since ontogenetic studies of avian development in representative orders are essential for addressing micro and macroevolution questions (Nagai et al., 2011), results of this research allow the standard identification of stages in different species of parrots, recognize similarities and differences between precocial (the chicken) and altricial species (*Myiopsitta*), and provide planning data for future studies, such as ossification sequences, myogenesis of the mandibular muscles, and brain development.

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