Ontogeny, Phylogeny, and Morphology in Anuran Larvae: Morphometric Analysis of Cranial Development and Evolution in Rana Tadpoles (Anura: Ranidae)

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ABSTRACT Comparative studies of chondrocranial morphology in larval anurans are typically qualitative in nature, focusing primarily on discrete variation or gross differences in the size or shape of individual structures. Detailed data on chondrocranial allometry are currently limited to only two species, Rana sylvatica and Bufo americanus. This study uses geometric morphometric and multivariate statistical analyses to examine interspecific variation in both larval chondrocranial shape and patterns of ontogenetic allometry among six species of Rana. Variation is interpreted within the context of hypothesized phylogenetic relationships among these species. Canonical variates analyses of geometric morphometric datasets indicate that species can be clearly discriminated based on chondrocranial shape, even when whole ontogenies are included in the analysis. Ordinations and cluster analyses based on chondrocranial shape data indicate the presence of three primary groupings (R. sylvatica; R. catesbeiana + R. clamitans; and R. palustris + R. pipiens + R. sphenocephala), and patterns of similarity closely reflect phylogenetic relationships. Analysis of chondrocranial allometry reveals that some patterns are conserved across all species (e.g., most measurements scale with negative allometry, those associated with the posterior palatoquadrate tend to scale with isometry or positive allometry). Ontogenetic scaling along similar allometric trajectories, lateral transpositions of individual trajectories, and variable allometric relationships all contribute to shape differences among species. Overall patterns of similarity among ontogenetic trajectories also strongly reflect phylogenetic relationships. Thus, this study demonstrates a tight link between ontogeny, phylogeny, and morphology, and highlights the importance of including both ontogenetic and phylogenetic data in studies of chondrocranial evolution in larval anurans. J. Morphol. 264:34–52, 2005. © 2005 Wiley-Liss, Inc.

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The fundamental contribution of ontogenetic processes to morphological variation within and among species has long been recognized (Thompson, 1917; Huxley, 1932; de Beer, 1937; Gould, 1977; Alberch et al., 1979; Reilly et al., 1997). Allometry, the differential scaling of individual anatomical components with overall body size, can have a profound impact on the shape of complex morphological structures (Huxley, 1932; Gould, 1966; Calder, 1984; Schmidt-Nielsen, 1984; Emerson and Bramble, 1993; Brown et al., 2000). Thus, knowledge of the relationship between morphological parameters and body size can provide valuable information regarding the developmental origins of morphological variation among species, particularly in groups where variation in body size is pronounced (Shea, 1983, 1985a). Larval anurans are particularly amenable to studies of allometry because they are readily available, easily reared, and typically undergo a dramatic increase in body size over a relatively short period of time. Despite these advantages, knowledge of the contribution of allometry to morphological change over the course of larval ontogeny in anurans remains limited (but see Wassersug and Hoff, 1979, 1982; Seale, 1982; Emerson, 1986; Strauss and Altig, 1992; Hall and Larsen, 1998; Larson, 2002, 2004).

The larval anuran chondrocranium is a complex structure that has been shown to vary considerably in morphology, even among closely related species (Haas, 1995; Wild, 1997, 1999; Haas and Richards, 1998; Larson and de Sá, 1998), and recent research suggests that there may be considerable phylogenetic signal in chondrocranial datasets (Haas, 1996, 2003; Larson and de Sá, 1998; Maglia et al., 2001; Puigener et al., 2003). Furthermore, given that much of the chondrocranium is intimately associated with muscles involved in gill irrigation and/or feeding, the shapes of chondrocranial cartilages provide valuable ecomorphological data that can be useful in identifying adaptational trends among larval anuran ecotypes (Gradwell, 1968, 1972a,b; Wassersug and Hoff, 1979; Satel and Wassersug, 1981; Haas and Richards, 1998; Larson and Reilly, 2003). However, despite the recent accumulation of compara-
tive data on qualitative/discrete variation in aspects of larval cranial morphology, quantitative studies investigating more subtle variation in chondrocranial shape are rare (but see Larson, 2004). Furthermore, data on chondrocranial growth allometry in larval anurans are limited to only two species (*Rana sylvatica*: Larson, 2002; and *Bufo americanus*: Larson, 2004), thus limiting our understanding of how ontogenetic processes contribute to variation in terminal larval shape among species. Such knowledge is essential to understanding the origin of evolutionary patterns of shape variation in these animals.

Available research indicates that although subtle phylogenetic patterns are apparent, qualitative variation in chondrocranial morphology among larval species of the genus *Rana* is limited (Parker, 1871, 1881; Gaupp, 1893; Stone, 1929; de Beer, 1937; Pusey, 1938; de Jongh, 1968; Kemp and Hoyt, 1969; Gradwell, 1972a; Plasota, 1974; Alley, 1989; Larson, in prep). Despite this, morphometric studies have demonstrated variation in external morphology in larval *Rana* (Korky, 1978; Hillis, 1982; Jennings and Scott, 1993), and quantitative ontogenetic data indicate that chondrocranial growth in *R. sylvatica* is characterized by significant shape change, with a distinct regional contrast in scaling patterns (Larson, 2002). In this species, measurements of the palatoquadrate and associated structures (e.g., the muscular process), which serve as insertion sites for key muscles involved in feeding and gill irrigation (Gradwell, 1968, 1972a,b; Wassersug and Hoff, 1979; Satel and Wassersug, 1981; Larson and Reilly, 2003), tend to scale with isometry or positive allometry. In contrast, most remaining measurements (e.g., length of otic capsules, braincase; measurements in the oral region) scale with negative allometry. Unfortunately, given the lack of comparative ontogenetic data, the contribution of these allometric patterns to interspecific morphological variation within *Rana*, or among anuran larvae in general, remains unclear.

This study employs recently developed morphometric methods (Bookstein, 1991; Rohlf and Marcus, 1993; Rohlf et al., 1996) to investigate the relationships between ontogeny, phylogeny, and morphology in six species of the genus *Rana* with a focus on the larval chondrocranium. This genus is particularly suitable for such a study given that data on phylogenetic relationships among species of *Rana* are available (e.g., Fig. 1; Hillis et al., 1983; Hillis and Davis, 1986), thus allowing interpretation of interspecific variation in a phylogenetic context. The primary goals of this study are: 1) to quantitatively compare chondrocranial shape among larval *Rana* using geometric morphometric methods; 2) to analyze and compare patterns of chondrocranial allometry among these species; 3) to determine whether patterns of similarity in chondrocranial shape and/or ontogenetic allometry coincide with expectations based on hypothesized phylogenetic relation-

ships among species; and 4) to determine the contribution of allometric growth to interspecific shape variation among *Rana* larvae.

**MATERIALS AND METHODS**

**Specimens Examined**

A total of 437 specimens (Appendix) representing six species of North American *Rana* were processed for quantitative analyses of chondrocranial anatomy; species examined include *Rana catesbeiana, R. clamitans, R. palustris, R. pipiens, R. sphenophthalmus, and R. sylvatica*. Phylogenetic relationships among these species, along with the Eurasian *Rana temporaria*, are depicted in Figure 1 (based on data in Hillis et al., 1983; Hillis and Davis, 1986). This sample includes some specimens examined in other studies (e.g., Larson, 2002); these are reanalyzed here to accommodate the inclusion of additional measurements.

Egg masses and/or larvae were either field-collected or obtained from commercial sources (see Appendix), and larvae were either subsequently reared in the laboratory or immediately euthanized (MS-222, Argent Chemical Laboratories, Redmond, WA) and preserved in formalin. Lab-reared tadpoles were fed commercial aquarium food ad libitum and housed at room temperature (20–23°C); specimens were periodically euthanized and preserved in order to obtain a complete developmental series for each species. All specimens were staged (Gosner, 1960), cleared, and double-stained for bone and cartilage using a modification of the technique of Dingekrus and Uhler (1977). Calipers were used to record both total length and snout–vent length (SVL; nearest 0.01 mm) for each individual. Sets of specimens for each species encompass nearly the entire larval period, with Gosner Stages 27–39 typically represented by at least one specimen each. Although a few specimens at Gosner Stages 25–26 were analyzed, cartilage in earlier developmental stages typically does not stain well with Alcian Blue. Later stages (>Stage 39) were not included to avoid potentially confounding effects of morphological changes associated with metamorphosis. Chondrocranial terminology follows Cannatella (1999).

**Data Acquisition**

**Image capture.** Dorsal views of the chondrocrania of all specimens (with a 1-mm scale bar) were photographed with a Panasonic WV-BL200 BW CCD video camera mounted on a Nikon SMZ-U Zoom 1:10 dissecting microscope. Specimens were oriented so that the palatoquadrate would lie in the plane of the

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**Fig. 1.** Phylogenetic relationships among the six species of North American *Rana* examined here, as well as the Eurasian *Rana temporaria*. The topology is based on data presented in Hillis et al. (1983) and Hillis and Davis (1986). *Rana sylvatica* has not been assigned to a species group.

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For allometry analyses, a total of 15 linear measurements were taken from chondrocrania (Fig. 2A) by calculating distances between Cartesian coordinates obtained from a set of landmarks digitized on the chondrocranium images (tpsDIG32 v1.33; Rohlf, 2003b). Chondrocranial measurements (Fig. 2A) are: 1) distance between the articulations of the suprarostral cartilages to the trabecular horns (SRA; a measure of gape width); 2) distance between the lateral corners of the palatoquadrate articular processes (PAQO; a rough measure of the distance between the articulations of Meckel’s cartilages to the palatoquadrate cartilages); 3) distance between the medial corners of the palatoquadrate articular processes (PAQI); 4) the lateral length of the palatoquadrate articular process (PAQL); 5) anterior width of the palatoquadrate articular process (PAQW); a rough measure of the distance between the articulations of Meckel’s cartilages to the palatoquadrate cartilages); 6) distance between the medial corners of the palatoquadrate articular processes (PAQI); 7) the lateral length of the palatoquadrate articular process (PAQL); 5) anterior width of the palatoquadrate articular process (PAQW); 6) width of the muscular process of the palatoquadrate (MPW; an estimate of the width of the orbitohyoideus muscle, the primary buccal depressor); 7) minimum width of the ethmoid plate posterior to the confluence of the trabecular horns (EPW); 8) distance between the braincase and the most lateral margin of the subocular bar of the palatoquadrate (BCPQ); 9) the distance between the braincase and the most lateral margin of the subocular bar of the palatoquadrate; 10) distance between the braincase and the most lateral margin of the subocular bar of the palatoquadrate; 11) distance from the confluence of the trabecular horns to the level of their anterior margin (THL); 12) proximal trabecular horn width (THW); 13) width of the braincase measured midway between the attachments of the anterior quadratoarticular commissure and the ascending process to the braincase (BCW); 14) length of the braincase from the posterior margin of the otic capsules to the confluence of the trabecular horns (BCL); and 15) maximum length of the otic capsule (OCL).

Measurements for allometry analyses were chosen to describe both the general shape of the chondrocranium (e.g., length and width of the braincase, length of otic capsules) and the shape of functionally important structures (e.g., muscle insertion sites, distance between jaw articulations, distance between ceratohyal articulations). In addition to chondrocranial measurements, SVL was included in allometry analyses as a measure of overall body size (see Larson, 2002). All measurements were log10-transformed prior to being input into statistical analyses.

For comparative analyses, a total of 15 landmarks (Fig. 2B) were digitized on one side of the chondrocranium of each specimen (tpsDIG32 v1.33; Rohlf, 2003b). Eleven of these landmarks correspond to those employed by Larson (2002), whereas four additional landmarks are included in the present analyses (Fig. 2B). Landmarks were chosen based on their ease of identification in all specimens, their ability to cover the entirety of the geometric form, and their ability to describe functionally important regions of the chondrocranium. Landmark configurations for each specimen were scaled to unit (=1) centroid size. Centroid size is defined as the square root of the sum of the squared distances from each landmark to the centroid of that landmark configuration. Landmark configurations for each specimen were subsequently superimposed and aligned using the generalized orthogonal least-squares Procrustes method to produce a consensus (tangent) configuration (tpaRelw v1.29; Rohlf, 2003a). Partial warp (n = 2p – 6 = 24, where p = # landmarks) and uniform component (n = 2; Bookstein, 1996) scores were obtained for each
specimen using standard geometric morphometric procedures (Bookstein, 1991, 1996; Rohlf, 1993; Rohlf et al., 1996; Larson, 2002), yielding a total of 26 variables.

Comparative Analyses

Terminal shape analysis. To minimize shape changes associated with ontogenetic growth, and to focus on later stages typically examined in comparative studies of chondrocranial anatomy, comparative analyses focus on a subset of specimens from the Appendix (“terminal shape” sample, n = 206) that approximates the terminal shape of the chondrocranium for larvae of each species (i.e., large, typically late-stage specimens). Previous research suggests that size is a better indicator of chondrocranial shape than Gosner (1960) stage (Larson, 2002). Since shape varies continuously with size during the larval period, and size varies considerably among species of *Rana*, it is difficult to identify a single stage or size at which interspecific comparisons should be made (Larson, 2002). Thus, to balance the need to minimize growth-related shape variation while maintaining adequate sample sizes for interspecific comparisons, subset of specimens for each species were chosen by plotting SVL against Gosner stage and selecting a threshold size above which specimens would be examined. In such plots, SVL typically plateaus at later Gosner stages (mid to late 30s), and threshold size was intended to capture specimens roughly around this plateau region. Threshold SVL and sample sizes for each species in the terminal shape dataset are: *Rana catesbeiana* (24 mm, n = 28), *R. clamitans* (20 mm, n = 38), *R. palustris* (16 mm, n = 34), *R. pipiens* (18 mm, n = 38), *R. sphenophthalm* (15 mm, n = 33), and *R. sylatica* (11 mm, n = 35). Most specimens represent Gosner Stages 33–39, although a few large specimens at earlier stages are also included. To further account for potential intraspecific variation, I examined field-collected samples from multiple geographic localities as well as specimens reared in the lab for most species (Appendix).

The geometric morphometric dataset (i.e., matrix of partial warp and uniform component scores for each specimen) for the terminal shape sample was analyzed using canonical variates analysis (NCSS; Hintze, 2001). Canonical variates analysis (CVA) calculates the linear combination of variables that maximally separates a priori defined groups (i.e., species in the present analyses; Manly, 1986). Thus, CVA is an effective tool for examining the degree to which these species can be distinguished on the basis of chondrocranial shape data. Furthermore, by plotting coefficients on the CV axes it is possible to assess patterns of similarity in shape among the groups. To allow interpretation of shape variation along the CV axes, partial warp and uniform component scores were regressed against the CV axes in the program tpsRegr (v1.26; Rohlf, 2002a), and thin-plate spline deformation grids and vector plots representing positive and negative deviations along each CV axis were examined. Mahalanobis distances (d^2) were also calculated between group means obtained from the CVA ordination on the terminal shape dataset.

To further investigate the correspondence between the degree of morphological similarity among species and phylogenetic relationships, consensus landmark configurations for the terminal shape samples of each species were computed in tpsRelw (v1.29; Rohlf, 2003a). A consensus configuration was also computed for a sample of eight specimens of *Rana temporaria* (SVL range = 11.7–14.1 mm) from Erfurt, Germany. *Rana temporaria* has been used as an outgroup in previous studies of North American *Rana* phylogeny (Hillis and Davis, 1986). Due to the small sample available for this species, it is only included in this single analysis. I computed Procrustes distances between consensus configurations pairwise between species. Procrustes distance is calculated as the square root of the sum of squared differences between the positions of corresponding landmarks in scaled and superimposed landmark configurations. Morphological phenograms based on both Procrustes distances and Mahalanobis distances between group means from the CVA of the terminal shape dataset were computed using the UPGMA clustering algorithm. Analyses of both distance matrices using the UPGMA procedure resulted in cophenetic correlations of 0.87 between the original distances and those that result from the cluster configuration. These phenograms are only intended to depict similarity in chondrocranial shape among the species.

Whole ontogeny analysis. To assess the degree to which ontogenetic changes might affect the ability to distinguish species based on chondrocranial shape, I analyzed the entire set of 437 specimens (see Appendix), including nearly complete larval ontogenetic series for each species. This sample will be referred to as the “whole ontogeny” sample. The geometric morphometric dataset for this sample was also analyzed using canonical variates analysis. The sole goal of this analysis was to determine whether these species can be distinguished on the basis of chondrocranial shape even when specimens representing nearly the whole larval ontogeny are included.

Analysis of Allometric Growth

Sets of lab-reared specimens (see above) for each species were examined to assess the relationship between body size and chondrocranial shape. Specimens examined in the analysis of allometry are indicated in boldface in the Appendix.

Principal components analysis (PCA) was used to independently analyze covariance matrices of the 16 log10-transformed linear measurements for each species. In strongly size-structured datasets, the vector of coefficients on the first component (i.e., the first eigenvector) of a PCA can be used as a generalization of simple allometry (Jolicoeur, 1963; Shea, 1985b; Klingenberg and Spence, 1993; Klingenberg, 1996). In this sense, PC1 can be thought of as describing ontogenetic trajectories for each species (Klingenberg and Spence, 1993). For the present datasets, the first principal component explained >94% (99.1% in *Rana catesbeiana*, 99.3% in *R. clamitans*, 98.3% in *R. palustris*, 98.6% in *R. pipiens*, 97.8% in *R. sphenophthalm*, and 94.2% in *R. sylatica*) of the total variance in each species, and the coefficients on the first eigenvector were of similar sign and magnitude, indicating that these data are suitable for such an analysis.

To determine scaling relationships, the ratio of coefficients on the first principal component (PC1) corresponds to (but does not necessarily equal) the coefficient that would be obtained if those same variables were regressed against one another in a typical bivariate allometry analysis (Shea, 1985b; Klingenberg, 1996). Under a null hypothesis of isometric growth relative to overall size as defined by the first PC axis, it is expected that all coefficients on PC1 would be equal and have the value 1/√p, where p represents the number of variables in the analysis (e.g., 1/√16 = 0.25 in the present analyses). I used a SAS/IML (SAS v. 8.2, SAS Institute, Cary, NC) bootstrap procedure (PCAs on 1,000 samples randomly drawn with replacement from each species dataset) to compute standard errors and confidence intervals for PC1 coefficients (Klingenberg, 1996). Confidence limits that include 0.25 indicate isometry relative to the overall size variable defined by PC1; those above 0.25 indicate positive allometry, whereas those below 0.25 indicate negative allometry. Scaling coefficients are also calculated for each variable against SVL, a standard larval size measurement, by dividing the coefficient for each variable by the coefficient for SVL. Confidence limits for scaling coefficients relative to SVL are determined by dividing the respective upper (UCL) or lower (LCL) confidence limit for each variable by the PC1 coefficient for SVL. Since all variables are length measurements, confidence limits that include 1.0 indicate isometric scaling; those above or below 1.0 indicate positive and negative allometry respectively.

To compare vectors of PC1 coefficients among species, I calculated angles between the PC1 axes pairwise between species (Klingenberg, 1996). This is done by multiplying corresponding PC coefficients for a pair of samples, summing results across all coefficients, and calculating the inverse cosine of the value obtained. These angles provide an estimate of overall similarity of scaling patterns/ontogenetic trajectories among species; an angle of zero would indicate that vectors of coefficients
are identical. Additionally, the vectors of PC1 coefficients for each species were analyzed using a second principal components analysis, and scores on the first two PC axes were plotted to provide a graphical comparison of ontogenetic trajectories among these six species (Klingenberg and Froese, 1991; Klingenberg and Spence, 1993). I also included 100 bootstrap estimates of the vectors of PC1 coefficients for each species in this analysis to assess the relative stability of positions of species in the multivariate space defined by the first two PC axes.

RESULTS

For comparative purposes and to facilitate visualization of interspecific and ontogenetic patterns discussed below, dorsal views of the chondrocrania of small, early stage and large, late stage specimens of all six species are provided in Figure 3.
Whole Ontogeny Analysis

Canonical variates analysis (CVA) of the whole ontogeny dataset (n = 437) indicates significant differences in chondrocranial shape among the six species (Wilks’ Lambda = 0.000374, P < 0.0001). Classification of specimens by placing them with the group mean to which they exhibited the smallest generalized distance (Mahalanobis $d^2$) yielded a 99.5% correct assignment (only two specimens were misclassified). Graphical plots of CV axes are provided (Fig. 4A–D) to allow visualization of the relative positions of specimens representing each specie.
cies in CVA space. A plot of the first two CV axes (Fig. 4A), which together explain 73.8% of the total variation in the whole ontogeny dataset, reveals three distinct clusters corresponding to: 1) an isolated cluster of specimens of *Rana sylvatica*; 2) a cluster corresponding to specimens of *R. palustris, R. pipiens, and R. sphenocephala*; and 3) a cluster corresponding to specimens of *R. catesbeiana* and *R. clamitans*. A plot of CV2 vs. CV3 indicates separation of *R. catesbeiana*, *R. clamitans*, and *R. sylvatica* from the remaining species, with some separation among members of the *R. pipiens* complex along CV3 (Fig. 4B). Canonical variate 4 contrasts *R. catesbeiana* and *R. sphenocephala* (specimens scoring high and positive) with remaining specimens (Fig. 4C). Canonical variate 5, in turn, partially discriminates *R. sphenocephala* (scoring low and negative; Fig. 4D) from all other taxa.

**Terminal Shape Analysis**

Canonical variates analysis of the terminal shape dataset (n = 206) also indicates significant differences in chondrocranial shape among the six species (Wilks’ Lambda = 0.00008, P < 0.0001), and classification of specimens as described above yields a 100% correct assignment. A plot of the first two CV axes (Fig. 5A), which together explain 73.2% of the total variation in the dataset, indicates groupings similar to those described above for the whole ontogeny dataset. However, CV2 does provide better separation of *Rana pipiens* from *R. palustris* and *R. sylvatica*, and *R. catesbeiana* from *R. clamitans*. A plot of CV2 vs. CV3 (Fig. 5B) indicates clear separation among species, with the exception of *R. clamitans* and *R. sylvatica*, which overlap each other slightly. A plot of CV3 vs. CV4 (Fig. 5C) clearly separates out *R. palustris* and *R. catesbeiana* from each other, and from the remaining species. A scatterplot of canonical variates 4 and 5, in turn, discriminates *R. sphenocephala* (Fig. 4D) from all other taxa.

Vector plots depicting shape change along the first two CV axes from the terminal shape dataset are presented in Figure 6. Plots of remaining axes are not presented, but shape change along these axes, ascertained by examining vector plots and thin-plate spline grids, is described below. Specimens scoring high and positive on CV1 (i.e., *Rana sylvatica*; Fig. 6B) are characterized primarily by: a more posterior attachment of the larval otic process to palatoquadrate (landmark 9); a shorter, more medially angled palatoquadrate articular process (landmarks 4, 5, and 6); and a wider muscular process (landmarks 6 and 8) with a more anteromedially located anterior margin (landmark 6). Species scoring lower on CV1 (particularly *R. catesbeiana* and *R. clamitans*; Fig. 6A) exhibit basically the opposite of these conditions, and I therefore only describe shape variation in one direction along each axis.

Specimens scoring positively on CV2 (i.e., *Rana clamitans, R. sylvatica*, most *R. catesbeiana*; Fig. 6D) are primarily characterized by: a more anteriorly located dorsal tip of the muscular process (landmark 13); a slight contrast in the positions of landmarks 6 (located more laterally) and 8 (located more medially), which is associated with a clockwise twist in the orientation of the muscular process; relatively narrower trabecular horns (landmarks 2 and 3); a shorter palatoquadrate articular process (landmarks 4 and 5); and longer otic capsules (landmarks 11 and 12). Specimens scoring high and positive on CV3 (i.e., *R. palustris*) are characterized primarily by particularly narrow muscular processes (landmarks 6 and 8) with the dorsal tip located further posterolaterally (landmark 13). Specimens with high, positive scores on CV4 (*R. catesbeiana*, many *R. palustris*) also have narrow muscular processes with more lateral tips, although the decrease in width is primarily associated with a more anterior position of landmark 8 in this case rather than a convergence of landmarks 6 and 8 as on CV3. High scores on CV4 also indicate a further posterior extension of the attachment of the larval otic process to the otic capsule (landmark 10). Finally, specimens scoring low and negative on CV5 (i.e., *R. sphenocephala*) are characterized by: a more anterior location of landmark 1, resulting in a short anterior extension of the trabecular horns; a shorter palatoquadrate articular process that is oriented further laterally (landmarks 4 and 5); and a more medial location of the lateral margin of the palatoquadrate (landmarks 6–8).

Procrustes distances calculated between mean landmark configurations for the six North American *Rana*, plus the Eurasian *R. temporaria*, indicate three primary groupings (Table 1). First, *R. sylvatica* and *R. temporaria* exhibit a lower distance (0.040) to each other than either does to any other taxon; distances between *R. sylvatica* and all other North American taxa are relatively high (0.052–0.077). Second, *R. catesbeiana* and *R. clamitans* exhibit a lower distance (0.037) to each other than either does to any other taxon (next closest is 0.045 between *R. clamitans* and *R. sphenocephala*). Finally, distances among *R. palustris, R. pipiens,* and *R. sphenocephala* are low and similar (0.037–0.042). Among these three main groups, distances are lower between the *R. catesbeiana + R. clamitans* group and *R. palustris + R. pipiens + R. sphenocephala* group, with the *R. sylvatica + R. temporaria* group exhibiting lower distances to members of the latter grouping. Although *R. temporaria* is not included, Mahalanobis distances (d²) calculated between group means from the canonical variates analysis of the terminal shape dataset present a similar pattern of distances among the North American species (Table 1).
Patterns of similarity based on both Procrustes and Mahalanobis distances are summarized by the UP-GMA phenograms in Figure 7A,B. These phenograms indicate groupings similar to those described above. The only difference between these phenograms, with the exception of the absence of *Rana temporaria* in the analysis based on Mahalanobis distances, is in the placement of *R. palustris*. The Procrustes distance phenogram clusters *R. palustris* with *R. pipiens* (Fig. 7A), whereas the Mahalanobis distance phenogram clusters *R. palustris* with *R. sphenoecephala* (Fig. 7B).

**Allometry Analysis**

Results of independent principal components analyses indicate considerable allometric growth in
all species relative to overall size as defined by the first PC axis (Table 2). Examination of scaling coefficients for chondrocranial variables relative to SVL (Table 3) indicates that although considerable variation is present, most measurements scale with negative allometry in these species (9/15 measurements in *Rana catesbeiana* and *R. sylvatica*; 11/15 in *R. clamitans*; 12/15 in *R. palustris, R. pipiens*, and *R. sphenocephala*).

Measurements associated with the braincase and otic capsules (BCW, BCL, OCL), articular process length (PAQL), the width of the ethmoid plate (EPW), and the distance between the ceratohyal articulation points (CAW) all scale with negative allometry in each of the six species (Table 3). However, some variability is apparent in the magnitude of the scaling coefficients for these measurements. For example, articular process length (PAQL) scales with slight negative allometry in *Rana catesbeiana* and *R. sylvatica* (scaling coefficient $\alpha = 0.928$) and *R. clamitans* ($\alpha = 0.963$), greater negative allometry in *R. palustris, R. pipiens*, and *R. sphenocephala* ($\alpha = 0.820 – 0.860$), and with the lowest coefficient in *R. sylvatica* ($\alpha = 0.723$). Scaling coefficients for ceratohyal articulation width (CAW), braincase width (BCW), and braincase length (BCL) are higher and similar.

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**TABLE 1.** Procrustes distances (lower left) and Mahalanobis distances (upper right) calculated pairwise between species

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<td>0.067</td>
<td>0.061</td>
<td>0.054</td>
<td>0.040</td>
<td>0</td>
</tr>
</tbody>
</table>

Procrustes distances are calculated between consensus landmark configurations from the terminal shape datasets for each species, as well as for an outgroup taxon, *Rana temporaria*. Mahalanobis distances are calculated between group means obtained from a canonical variates analysis of the terminal shape dataset. *Rana temporaria* was not included in the canonical variates analysis due to the small size of the available sample, and Mahalanobis distances are therefore not reported for this species.
among *R. catesbeiana*, *R. clamitans*, *R. sylvatica*, and lower and similar among *R. palustris*, *R. pipiens*, and *R. sphenocephala* (Table 3). The scaling coefficient for otic capsule length (OCL) is slightly lower in *R. sylvatica* ($\alpha = 0.675$) than in other species.

The remaining measurements exhibit variability in allometric patterns (Table 3). The distance between the suprarostal articulations (SRA) scales isometrically in *Rana catesbeiana* and *R. palustris*, and with negative allometry in all other species. The distances between the outer and inner corners of the palatoquadrate articular processes (PAQO, PAQI) scale with positive allometry in *R. catesbeiana* and *R. clamitans*, and with negative allometry in other species. The width of the palatoquadrate articular process (PAQW) scales isometrically in *R. catesbeiana* and *R. sylvatica*, and with negative allometry in all other species (Table 3). Muscular process width (MPW) scales with positive allometry in *R. sylvatica* ($\alpha = 1.081$), and with negative allometry in all other species, although in *R. catesbeiana* it is nearly isometric ($\alpha = 0.966$). Both measurements associated with the lateral extension of the palatoquadrate from the braincase (BCPQ, MPQ) scale with slight positive allometry in *R. catesbeiana*, *R. clamitans*, and *R. sylvatica*, and except for MPQ in *R. palustris* (slight negative allometry), isometry in members of the *R. pipiens* complex. Anterior extension of the trabecular horns (THL; Table 3) scales isometrically in *R. sylvatica* and *R. pipiens*, with very slight negative allometry in *R. catesbeiana*, *R. clamitans*, and *R. palustris*, and with greater negative allometry in *R. sphenocephala* ($\alpha = 0.855$). Proximal trabecular horn width (THW) scales isometrically in *R. sphenocephala*, with slight negative allometry (*R. catesbeiana*, *R. palustris*, *R. pipiens*), or with more pronounced negative allometry as in *R. clamitans* ($\alpha = 0.800$).

Although angles calculated between the PC1 axes (i.e., first eigenvectors) computed for the six North American species are all fairly low (<7.65°), patterns of similarity among species suggest three primary groupings (Table 4). First, angles between *Rana sylvatica* and all other taxa are relatively high (5.90–7.65°), indicating that allometric growth patterns in this species are distinct. Second, the angle between *Rana catesbeiana* and *R. clamitans* (3.29°) is lower than that between either of these species and any other taxon (nearest is 3.43° between *R. catesbeiana* and *R. sphenocephala*). Finally, angles between PC1 axes for *R. palustris*, *R. pipiens*, and *R. sphenocephala* are low and similar (2.59–3.24°). Among these three main groups, angles are lower between the *R. catesbeiana* + *R. clamitans* group and *R. palustris* + *R. pipiens* + *R. sphenocephala* group than between either of these groups and *Rana sylvatica*. A plot of the first two axes (Fig. 8) from a principal components analysis of the vectors of PC1 coefficients (Table 2; with 100 bootstrap estimates

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**Fig. 7.** UPGMA phenograms indicating patterns of similarity in chondrocranial shape and among ontogenetic trajectories of *Rana* species. **A:** Phenogram based on clustering of a matrix of Procrustes distances ($d^2$) between consensus forms for each species computed from specimens in the terminal shape dataset. A consensus of eight specimens of *R. temporaria* is also included in this analysis. This phenogram provides an indication of patterns of similarity of chondrocranial shape among species. **B:** Phenogram based on clustering of the matrix of Mahalanobis distances ($d^2$) between group (=species) means obtained from a canonical variates analysis on the matrix of partial warp and uniform component scores for the terminal shape dataset. The sample of *R. temporaria* larvae was not sufficient to be included in this analysis. This phenogram also provides an indication of patterns of similarity of chondrocranial shape among species. **C:** Phenogram based on clustering of the matrix of angles between vectors of PC1 coefficients obtained from independent principal components analyses of 16 linear measurements for each species. The sample of *R. temporaria* larvae was not sufficient to be included in this analysis. This phenogram provides an indication of overall similarity of ontogenetic trajectories among species.
for each) for those suggested by examining angles between vectors of PC1 coefficients. A UPGMA phenogram summarizing patterns of similarity in ontogenetic trajectories is presented in Figure 7C and further corroborates these groupings.

**DISCUSSION**

The results presented above demonstrate that: 1) the six species of *Rana* examined here can be clearly discriminated morphometrically based on terminal chondrocranial shape, and also when specimens representing whole larval ontogenies are examined; 2) patterns of similarity in chondrocranial shape reflect phylogenetic relationships among these species; 3) most chondrocranial measurements exhibit non-isometric growth; 4) overall patterns of ontogenetic allometry, although variable among species, also directly reflect phylogenetic relationships; and 5) ontogenetic processes (i.e., scaling patterns) have a direct influence on patterns of interspecific shape variation. These results are discussed in more detail below.

**Patterns of Variation in Chondrocranial Shape**

The results of canonical variates analyses on both the whole ontogeny and terminal shape geometric morphometric datasets indicate that chondrocranial shape is clearly distinct among the six species of *Rana* examined here. This is exemplified by the relatively clear distinction among clouds of data points representing each species in CVA space (Figs. 4, 5), as well as the fact that only two specimens...
were misclassified in the analysis of the whole ontogeny dataset, and 100% were classified correctly in the analysis of the terminal shape dataset. Both of the misclassified specimens were members of the R. pipiens complex, and were misclassified as another member of the complex. Both were also among the smallest and earliest stage individuals examined for their respective species (R. pipiens, Stage 26, SVL = 5.9 mm, misclassified as R. sphenoecephala; R. palustris, Stage 26, SVL = 6.2 mm, misclassified as R. pipsiens). This indicates that small, very early stage larvae in closely related taxa may be more difficult to classify than larger, later stage individuals. Nonetheless, the ability to clearly discriminate species even when nearly whole larval ontogenies are included indicates that some of the distinguishing fea-

### TABLE 3. Scaling coefficients for chondrocranial variables relative to snout-vent length (SVL) as determined from independent principal components analyses on each species dataset

<table>
<thead>
<tr>
<th>Species</th>
<th>R. catesbeiana</th>
<th>R. clamitans</th>
<th>R. palustris</th>
<th>R. pipiens</th>
<th>R. sphenoecephala</th>
<th>R. sylvatica</th>
</tr>
</thead>
<tbody>
<tr>
<td>SRA</td>
<td>1.000</td>
<td>0.973</td>
<td>1.027</td>
<td>0.893</td>
<td>0.870</td>
<td>0.915</td>
</tr>
<tr>
<td>PAQQ</td>
<td>1.023</td>
<td>1.004</td>
<td>1.045</td>
<td>1.026</td>
<td>1.015</td>
<td>1.037</td>
</tr>
<tr>
<td>PAQI</td>
<td>1.053</td>
<td>1.027</td>
<td>1.080</td>
<td>1.070</td>
<td>1.052</td>
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<tr>
<td>PAQL</td>
<td>0.928</td>
<td>0.898</td>
<td>0.962</td>
<td>0.963</td>
<td>0.933</td>
<td>0.989</td>
</tr>
<tr>
<td>PAQW</td>
<td>0.970</td>
<td>0.947</td>
<td>1.000</td>
<td>0.900</td>
<td>0.874</td>
<td>0.933</td>
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<tr>
<td>MPW</td>
<td>0.966</td>
<td>0.932</td>
<td>0.992</td>
<td>0.848</td>
<td>0.811</td>
<td>0.878</td>
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<td>EPW</td>
<td>0.678</td>
<td>0.644</td>
<td>0.701</td>
<td>0.659</td>
<td>0.637</td>
<td>0.678</td>
</tr>
<tr>
<td>BCPQ</td>
<td>1.030</td>
<td>1.004</td>
<td>1.053</td>
<td>1.063</td>
<td>1.041</td>
<td>1.081</td>
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<tr>
<td>MPQ</td>
<td>1.053</td>
<td>1.008</td>
<td>1.083</td>
<td>1.119</td>
<td>1.070</td>
<td>1.170</td>
</tr>
<tr>
<td>CAW</td>
<td>0.962</td>
<td>0.951</td>
<td>0.970</td>
<td>0.952</td>
<td>0.941</td>
<td>0.963</td>
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<tr>
<td>THL</td>
<td>0.958</td>
<td>0.936</td>
<td>0.985</td>
<td>0.959</td>
<td>0.933</td>
<td>0.981</td>
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<tr>
<td>THW</td>
<td>0.909</td>
<td>0.867</td>
<td>0.958</td>
<td>0.800</td>
<td>0.767</td>
<td>0.841</td>
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<tr>
<td>BCW</td>
<td>0.792</td>
<td>0.769</td>
<td>0.818</td>
<td>0.756</td>
<td>0.733</td>
<td>0.781</td>
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<tr>
<td>BCL</td>
<td>0.951</td>
<td>0.932</td>
<td>0.966</td>
<td>0.911</td>
<td>0.900</td>
<td>0.922</td>
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<tr>
<td>OCL</td>
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<td>0.777</td>
<td>0.826</td>
<td>0.759</td>
<td>0.741</td>
<td>0.778</td>
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</tbody>
</table>

### TABLE 4. Angles between first eigenvectors (i.e., vectors of PC1 coefficients) obtained from independent principal components analyses of linear measurements for each species

<table>
<thead>
<tr>
<th>Species</th>
<th>R. catesbeiana</th>
<th>R. clamitans</th>
<th>R. palustris</th>
<th>R. pipiens</th>
<th>R. sphenoecephala</th>
<th>R. sylvatica</th>
</tr>
</thead>
<tbody>
<tr>
<td>R. catesbeiana</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>R. clamitans</td>
<td>3.29</td>
<td>0.00</td>
<td>3.98</td>
<td>4.96</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>R. palustris</td>
<td>3.83</td>
<td>4.52</td>
<td>2.59</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
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<tr>
<td>R. pipiens</td>
<td>3.43</td>
<td>4.97</td>
<td>2.81</td>
<td>3.24</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>R. sphenoecephala</td>
<td>6.24</td>
<td>7.65</td>
<td>7.23</td>
<td>5.90</td>
<td>6.21</td>
<td>0.00</td>
</tr>
<tr>
<td>R. sylvatica</td>
<td>6.24</td>
<td>7.65</td>
<td>7.23</td>
<td>5.90</td>
<td>6.21</td>
<td>0.00</td>
</tr>
</tbody>
</table>
tures of chondrocranial anatomy in these species are present at the earliest stages examined here. Furthermore, the overall clear discrimination among species on the basis of chondrocranial shape, particularly among members of the *R. pipiens* complex, which are highly conservative in external morphology (Wassersug, 1976; Korky, 1978; Hillis, 1982), points to the potential utility of geometric morphometric analysis of chondrocranial shape data as a taxonomic tool that can be used in species identification.

One of the benefits of using a geometric morphometric approach in concert with multivariate statistical analyses is that it allows for the identification of shape differences among species that may not be readily apparent in traditional comparative analyses of qualitative morphological variation. Comparative studies of chondrocranial anatomy in larval anurans have historically been largely qualitative (e.g., Haas, 1995, 2003; Fabrezi and Vera, 1997; Larson and de Sa, 1998; Haas and Richards, 1998; Maglia et al., 2001; Larson et al., 2003; Pu`gener et al., 2003), and while such studies are extremely valuable, it is possible that more subtle shape variation may be overlooked. For example, chondrocranial anatomy in *Rana* larvae is overall conserved, with very little discrete variation apparent (Larson, in prep). Whereas subtle variation in the position or size of certain structures can be observed (i.e., the position of attachment of the larval otic process to the palatoquadrate is distinct in *R. sylvatica*, Fig. 3L; Larson, in prep.), it is difficult to thoroughly and accurately analyze patterns of nondiscrete variation among a large number of specimens without a rigorous quantitative analysis.

Interpretation of shape variation implied by the CV axes allows identification of shape features that distinguish each of these taxa from one another (see Results for a detailed summary). As an example, CV1 clearly separates *Rana sylvatica* from the other species (Figs. 4A, 5A). Examination of shape variation along this axis for the terminal shape dataset corroborates the observation that the position of attachment of the larval otic process to the palatoquadrate is located further posteriorly in *R. sylvatica* (Fig. 6B). However, it also demonstrates that this species is distinguished by the presence of wider muscular processes, and shorter, more medially oriented palatoquadrate articular processes as compared to the other taxa (Fig. 6B). Such observations are difficult to make without a quantitative approach, and it is hoped that these results will not only spur quantitative analyses of chondrocranial morphology in additional groups of anurans, but also morphometric studies in other groups of animals that exhibit relatively little qualitative morphological variation.

**Chondrocranial Shape and Phylogeny**

In addition to distinguishing individual species, examination of the relative positions of clusters of data points corresponding to each species in ordinations allows for an assessment of the relative similarity of chondrocranial shape among these species. For example, scatter plots of scores on the first two CV axes from analyses of both the whole ontogeny and terminal shape datasets (Figs. 4A, 5A) indicate three primary clusters of points corresponding to the following groupings: 1) *Rana sylvatica*, 2) *R. catesbeiana* + *R. clamitans*, and 3) *R. palustris* + *R. pipiens* + *R. sphenoecephala*. These three groupings correspond exactly to the three primary groupings indicated by the hypothesized phylogenetic relationships among these species (Fig. 1; Hillis et al., 1983; Hillis and Davis, 1986). In fact, the only incongruence indicated by both the ordinations (Figs. 4A, 5A) and a phenogram computed based on Mahalanobis distances between species means (Fig. 7B) is greater similarity in chondrocranial shape between *R. palustris* and *R. sphenoecephala*; current hypotheses of phylogenetic relationships in *Rana* suggest a closer relationship between *R. pipiens* and *R. sphenoecephala* (Fig. 1; Hillis et al., 1983; Hillis and Davis, 1986).

A phenogram based on Procrustes distances between consensus landmark configurations for each species also matches the phylogeny for the genus remarkably well (Fig. 7A), although in this analysis *Rana pipiens* is more similar to *R. palustris*. Interestingly, inclusion of the Eurasian *R. temporaria* in this analysis indicates strong similarity in chondro-
cranial shape between this species and \textit{R. sylvatica}. The relationship of \textit{R. sylvatica} to other North American taxa has been the subject of considerable debate, with various authors grouping this species with either the eastern or western North American \textit{Rana} (Chantell, 1970; Wallace et al., 1973; Case, 1978; Farris et al., 1979, 1982; Post and Uzzell, 1981; Uzzell and Post, 1986). In a congruence tree based on allozymic, morphological, immunological, and rDNA restriction-site data, Hillis and Davis (1986) suggest that \textit{R. sylvatica} is most closely related to \textit{R. temporaria}, and that these two are most closely related to the \textit{R. boylii} group from the western United States. Although larval specimens from the \textit{R. boylii} group were not included here, chondrocranial shape data indicate a strong similarity between \textit{R. sylvatica} and \textit{R. temporaria}, potentially supporting a closer relationship between these taxa.

Taken together, the overall congruence of patterns of similarity in larval chondrocranial shape with hypothesized phylogenetic relationships based primarily on molecular/biochemical data suggests that the larval data contain considerable phylogenetic signal (Cole et al., 2002). Considerable debate exists regarding the potential and methodology for the direct inclusion of morphometric data in phylogenetic analyses (Fink and Zelditch, 1995; Adams and Rosenberg, 1998; Rohlf, 1998; Zelditch et al., 1998; MacLeod and Forey, 2002; Rohlf, 2002b). Despite this, the results presented here provide further support for the continued application of chondrocranial data to questions of anuran phylogeny.

Patterns of Chondrocranial Allometry

Available data on chondrocranial allometry in larval anurans are currently restricted to two species, \textit{Rana sylvatica} and \textit{Bufo americanus} (Larson, 2002, 2004). This study furthers knowledge of chondrocranial allometry in anurans by providing data on additional measurements in \textit{R. sylvatica}, as well as new data for five additional species of \textit{Rana}. Results presented here indicate that: 1) most chondrocranial measurements exhibit allometric growth relative to SVL in all species; 2) some measurements exhibit similar scaling relationships among groups of species, indicating the potential for interspecific shape differences resulting from ontogenetic scaling along common growth trajectories; and 3) some measurements exhibit variable scaling relationships among species, indicating that differing allometric growth patterns also contribute to interspecific shape variation.

Larson (2002) demonstrated that chondrocranial growth in \textit{Rana sylvatica} is highly allometric, with measurements associated with the posterior palatoquadrate tending to scale with isometry or positive allometry, and most remaining measurements (e.g., length of otic capsules, braincase; measurements in the oral region) scaling with negative allometry. These general patterns held for other species of \textit{Rana} examined here, as measurements associated with the braincase and otic capsules scaled with negative allometry, and those associated with the lateral extension of the posterior palatoquadrate scaled either isometrically or with positive allometry in all six species (Table 3). The only exception to this pattern was MPQ in \textit{R. palustris}, which scaled with slight negative allometry (\(\alpha = 0.961\)). As mentioned above, the posterior palatoquadrate serves as the insertion site for the primary muscles involved in jaw closure during feeding (Larson and Reilly, 2003), and the differential scaling observed in this region could be related to either the functional importance of this structure as a muscle insertion site or to the effects of forceful muscle contraction on cartilage growth. Since both the distance between the upper jaw articulations (SRA; a measure of gape width) and the distance between the ceratohyal articulations (CAW; a measure of buccal cavity width) scale with negative allometry in all species, which together would seemingly diminish filter feeding capabilities through ontogeny, allometric or proportional enlargement of the jaw closing muscles suggests increasing importance of rasping feeding as these animals grow. This observation is supported by experimental data indicating decreasing efficiency of filter feeding at later stages during larval ontogeny in anurans (Viertel, 1990, 1992).

Overall, most measurements in these species scale with negative allometry relative to SVL (Table 3). As an example, negative allometry for otic capsule length can easily be seen by observing the proportionally smaller otic capsules in late-stage specimens in Figure 3. Subgroups of species also often share very similar growth allometries for certain measurements (Table 3). Given that maximum SVL varies considerably among these species (e.g., \textit{Rana catesbeiana} larvae can reach a SVL three times that observed in the largest \textit{R. sylvatica} specimen examined here), species that share similar allometric trajectories for a given measurement will exhibit shape variation at differing terminal sizes that is a function of ontogenetic scaling along these trajectories (Gould, 1966; Shea, 1983, 1985a). An example is provided by examining braincase length (BCL) in \textit{R. catesbeiana} and \textit{R. sylvatica}, which scales with negative allometry of similar magnitude in these two species (\(\alpha = 0.951\) and 0.915, respectively). A plot of these two variables (Fig. 9A) demonstrates the differential growth of these two species along a shared allometric trajectory, with \textit{R. catesbeiana} larvae reaching considerably larger terminal sizes. A similar pattern is exhibited by the similar negative allometric scaling of the distance between the ceratohyal articulations (CAW) in members of the \textit{R. pipiens} complex (Fig. 9B), in which \textit{R. pipiens} extends slightly further along the shared trajectory. Although similarity of allometric patterns for subgroups of taxa is apparent for many measurements,
Fig. 9. Scatterplots of selected variables against SVL to demonstrate variation in allometric patterns observed among species. All variables are log_{10}-transformed. When present, lines represent least squares regression lines fitted to the data for each species.

Species Key
- \( R. \) catesbeiana
- \( R. \) clamitans
- \( R. \) palustris
- \( R. \) pipiens
- \( R. \) sphenopephala
- \( R. \) sylvatica
few measurements exhibited close similarity of scaling patterns among all species (Table 3). In fact, scaling coefficients for many measurements were quite variable among species. For example, muscular process width (MPW) scales with positive allometry in *Rana sylvatica* and negative allometry in all other species; the scaling coefficient for MPW was lowest in *R. palustris* (Table 3). A plot of MPW against SVL in these two species, along with *R. clamitans*, clearly demonstrates that muscular process width is very similar among these three species early in development, but due to varying allometric growth patterns, becomes proportionally larger in *R. sylvatica* and proportionally smaller in *R. palustris* later in development (Fig. 9C). This variation is borne out in the comparative analysis of the terminal shape dataset (Fig. 5), where *R. sylvatica* was discriminated along CV1 in part by having a wide muscular process (Fig. 6B), and *R. palustris* was discriminated along CV3 primarily by having a particularly narrow muscular process. As another example, the scaling coefficient for the length of the anterior extension of the trabecular horns (THL) was considerably lower in *R. sphenoecephala* (\( \alpha = 0.855 \)) than in other species (Table 3). A plot of this variable against SVL demonstrates how this allometric relationship results in differentiation of *R. sphenoecephala* from other members of the *R. pипiens* complex at larger sizes (Fig. 9D), and is manifested in the comparative analysis by discrimination of *R. sphenoecephala* along CV5 (Fig. 5D) due to the presence of a shorter anterior extension of the trabecular horns. Additional examples of how variable allometric patterns contribute to interspecific shape variation can be found by examining and comparing scaling coefficients in Table 3.

Other measurements exhibit more complex patterns among species. For example, scaling coefficients for articular process length (PAQL) are highly variable among species (Table 3). *Rana sylvatica* exhibits the lowest scaling coefficient for this variable (\( \alpha = 0.723 \)), which corresponds directly to the fact that the presence of short articular processes contributed to the discrimination of this species along CV1 (Figs. 5A, 6B). A plot of PAQL against SVL (Fig. 9E) clearly demonstrates greater negative allometry for this variable in *R. sylvatica*. However, it also shows that despite their lower scaling coefficients, members of the *R. pипiens* complex have proportionally longer articular processes than *R. clamitans* and *R. catesbeiana* at the smallest sizes examined here, but are comparable to *R. clamitans* and *R. catesbeiana* at larger sizes. This is an example of a set of scaling relationships where some species exhibit lateral transpositions (Shea, 1985b; Klingenberg and Spence, 1993) of growth trajectories (e.g., species in the *R. pипiens* complex), whereas others have similar start points (i.e., similar intercepts) but exhibit variation in the slope of the allometric relationship (e.g., compare *R. sylvatica* and *R. clamitans* in Fig. 9E). Similarly, the distance between the inner corners of the palatoquadrate articular processes (PAQL) exhibits complex patterns among *R. clamitans*, *R. sphenoecephala*, and *R. sylvatica* (Fig. 9F). Lateral transpositions of ontogenetic trajectories clearly separate these species early in development, with *R. sylvatica* have the longest distance and *R. clamitans* the shortest, but variable allometric patterns reverse this pattern later in larval ontogeny, and these two taxa are placed at the extremes along CV1 (Fig. 6A) in part due to variation in the position of the articular process (see landmarks 4, 5 in Fig. 6A,B).

Comparisons of ontogenetic trajectories as a whole reveal three primary clusters of species and indicate that ontogenetic patterns closely track phylogenetic relationships among these groups (Table 4, Fig. 8). In fact, a phenogram computed from the angles between vectors of PC1 coefficients (Fig. 7C) is largely congruent with the current phylogenetic hypothesis for these taxa (Fig. 1; Hillis et al., 1983; Hillis and Davis, 1986). Once again, the only disagreement is in the greater similarity between *Rana palustris* and *R. pипiens*. Furthermore, with the exception of the absence of *R. temporaria*, the phenogram based on similarity of ontogenetic trajectories is in complete congruence with that based on clustering of Procrustes distances (i.e., chondrocranial shape).

### Ontogeny, Phylogeny, and Morphology in *Rana* Larvae

The results of the analyses presented here demonstrate that although distinguishing morphological features are apparent at the earliest stages examined (Fig. 4), it is clear that ontogenetic patterns and interspecific morphological variation are directly associated, and that allometry can have a profound and complex impact on chondrocranial shape in larval anurans. Furthermore, patterns of similarity in both ontogeny and morphology, in turn, are highly correlated with phylogenetic relationships among these species. Taken together, these data provide compelling evidence for a tight link between ontogeny, phylogeny, and morphology in larval anurans of the genus *Rana*, and they highlight the role of development in contributing to interspecific morphological variation. Furthermore, they reiterate that the incorporation of both ontogenetic and phylogenetic information is both possible and essential when attempting to understand the evolution of complex and developmentally dynamic morphological structures such as the larval anuran chondrocranium.

### ACKNOWLEDGMENTS

I thank Robert Murphy of the Royal Ontario Museum, Ron Heyer of the Smithsonian Institution, and Alex Haas of the Biozentrum Grindel und Zo-
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## APPENDIX. List of specimens examined in this study

<table>
<thead>
<tr>
<th>Species</th>
<th>Collection locality/source</th>
<th>Sample size</th>
<th>Gosner stages</th>
<th>SVL range</th>
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</thead>
<tbody>
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<td><strong>Rana catesbeiana</strong></td>
<td>Long Lake, Harrison, ME</td>
<td>42</td>
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<td>Shade, Athens County, OH</td>
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<td><strong>Rana clamitans</strong></td>
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<td>Lake Hope, Vinton County, OH</td>
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<td>Jackson County, OH</td>
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<td>Minker’s Run, Nelsonville, OH</td>
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<td>Virginia (USNM #299871**)</td>
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<td><strong>Rana pipiens</strong></td>
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<td>71</td>
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<td>Haldimand, Ontario, Canada (ROM 11945*)</td>
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<td><strong>Rana sphenoecephala</strong></td>
<td>Charles D. Sullivan Co., Nashville, TN</td>
<td>71</td>
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<td><strong>Rana sylvatica</strong></td>
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<td>Fort A.P. Hill, Caroline County, VA</td>
<td>10</td>
<td>30–33, 35</td>
<td>10.8–14.2</td>
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</table>

*ROM = Royal Ontario Museum; **USNM = United States National Museum, Smithsonian Institution.

For comparative analyses of chondrocranial shape, all specimens listed were included in the whole ontogeny dataset, whereas a size-restricted subset was chosen for the terminal shape analysis (see text). Samples indicated in boldface were examined in analyses of chondrocranial allometry.