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New information on sexual dimorphism and allometric growth in *Keichousaurus hui*, a pachypleurosaur from the Middle Triassic of Guizhou, South China

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*Keichousaurus hui* is a small pachypleurosaur (Reptilia: Sauropterygia) from the Triassic of China. Many specimens of various growth stages are known, making them ideal for ontogenetic research. We report 22 new specimens from the Middle Triassic of Xingyi (Guizhou, south China), and combined their skeletal measurements with those from 85 published specimens to analyze the ontogenetic trajectory of sexual dimorphism. An Exploratory Factor Analysis suggests that the largest factors behind morphological disparity within the species are body size followed by gender. Sexual dimorphism is most clearly reflected in selected skeletal ratios that are more pronounced in males than in females. We found that the relative length of femur to body size was useful in gender identification, in addition to three ratios that are traditionally used, namely a distal expansion of the humerus relative to its shaft, humerus length relative to body size, and humerus length relative to femur length. Two distinctive patterns exist in allometric changes of these four ratios. The distal expansion of the humerus is exceptional in that it is equally pronounced in juvenile and adult males and therefore must have been fully established during embryonic growth. The other three features are not pronounced at birth size and subsequently become pronounced during postembryonic growth. However, males and females already show different growth trajectories at birth size even in these three. Therefore, the fate of sexually dimorphic features seems to have already been set during embryonic growth in *K. hui*.

Key words: Pachypleurosauria, Sauropterygia, *Keichousaurus hui*, allometry, sexual dimorphism, Triassic, China.

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Introduction

Different parts of the body may grow at various rates during ontogeny (i.e., allometric growth), modifying their proportions as a result. In addition, males and females of the same species often exhibit different growth trajectories, leading to sexual dimorphism (Chapman et al. 1997). A far larger proportion of extant species are sexually dimorphic than monomorphic (Fairbairn 1997; Kaliontzopoulou et al. 2007). These two aspects, allometric growth and sexual dimorphism, have been crucial in characterizing the ontogeny of a species. Analysis of allometry and sexual dimorphism are more complex for fossil animals. For instance, limitations from fossil preservation, such as small sample sizes and scar-
city of newborn and subadult specimens, add to the difficulties. In reptiles, sexual dimorphism is usually exhibited in soft tissues, such as the color of skin and head crest, which are rarely preserved in fossils.

Pachypleurosauria, a stem taxon of sauropterygians, is a member of Nothosauria (Rieppel 2000). It is different from Nothosauroidea based on a suite of features, including smaller size and a quadrate fossa behind the articular facet in the retroarticular process. Despite the challenges mentioned above, much work has been accomplished on the ontogeny of pachypleurosauris, thanks to the large number of well-preserved specimens. Several standards have been established in discriminating the genders in pachypleurosauris. For example, Sander (1989) proposed that sexual dimorphism in the Alpine genera *Neusticosaurus* and *Serpianosaurus* is found mainly in the morphometrics of the humerus, and he used the following ratios: distal width/minimal diameter of the humerus, humerus/femur length, humerus/trunk length, and humerus/standard length. Later, Lin and Rieppel (1998) introduced a set of similar ratios for gender identification and sexual dimorphism characterization in *Keichousaurus hui*. They found features of the humerus to be the ideal indicators of gender. The humerus of sex x, which later was identified as female based on gravid individuals (Cheng et al. 2004; Organ et al. 2009), tends to be more slender and shorter than that of sex y, with unpronounced ends both proximally and distally (Fig. 1). Further, Cheng et al. (2009) calculated threshold values between sexes x and y in three ratios: distal width/minimal diameter of humerus, humerus/femur lengths, and humerus/standard lengths, and used principal component analysis (PCA) and discriminant function analysis (DFA), which is usually a synonym of linear discriminant analysis (LDA) in deciphering genders. Though existing methods are useful to some extent, there are cases where they result in contradictory gender identifications for a single well-preserved specimen (especially for juveniles).

Sexual dimorphism in allometry has rarely been addressed in extinct taxa, but is known in many extant animals (Fairbairn 1997; Gregory 2004; Stoltz et al. 2005; Wooterspoon 2011). By analyzing sexual dimorphism alone, Cheng et al. (2009) concluded that sexual maturity was reached in males at approximately 126 mm snout-vent length (SVL) and 122 mm in females. But it is still unclear at what size *K. hui* starts to differentiate sexually. It was not clear if they have a special onset of sexual dimorphism during the juvenile period or they are different from birth. One way to solve this problem is to investigate allometric growth and sexual dimorphism simultaneously in a single analysis.

The purpose of our study is three-fold: first to scrutinize the published gender identification in *Keichousaurus hui*, second to test if sexual dimorphism is a major factor behind morphological disparity in the species, and third to clarify the allometry of sexually dimorphic characters in this species to identify the onset of sexual dimorphism. *K. hui* was chosen because we have ample samples that enable adequate statistical power to address the above questions. In addition, the
follow their fig. 1 that depicted the measurements. These two consistent with those of Cheng et al. (2009), although we tried to measure the body was computed and used in the analyses. After averaging, the orientation of exposure, i.e., dorsal or ventral, was available at http://app.pan.pl/SOM/app60-Xue_etal_SOM. The measurements were logarithmically transformed to base 10.

When the length of a body part exceeded the range of the calipers (150 mm), a ruler was used, and measurements then recorded to the nearest 0.1 mm. Some parts, such as the neck, trunk, and tail are not always preserved in a straight position. When the length of a body part exceeded the range of the calipers (150 mm), a ruler was used, and measurements then recorded to the nearest 0.1 mm. Some parts, such as the neck, trunk, and tail are not always preserved in a straight position.

Physical measurements.—All measurements were made with a pair of digital calipers with an accuracy of 0.01 mm, and a microscope was used for greater accuracy with small parts of the body (vertebrae, girdle bones, limbs, and skull). When the length of a body part exceeded the range of the calipers (150 mm), a ruler was used, and measurements then recorded to the nearest 0.1 mm. Some parts, such as the neck, trunk, and tail are not always preserved in a straight line, and in such cases, a cotton thread was aligned with the body parts and measured by the ruler later. Fourteen features were measured (see Table 1 in Supplementary Online Material available at http://app.pan.pl/SOM/app60-Xue_etal_SOM.pdf). The orientation of exposure, i.e., dorsal or ventral, was noted. The mean of measurements from both sides of the body was computed and used in the analyses. After averaging, measurements were logarithmically transformed to base 10.

Our measurements of skull and trunk lengths are inconsistent with those of Cheng et al. (2009), although we tried to follow their fig. 1 that depicted the measurements. These two characters were excluded from the analyses to avoid confusion. In addition, the femoral length of NMNS-kiko2004-01 is unusually short for the body size, so this specimen was excluded from those analyses that are relevant to femur. Similarly, NMNS-cyn2002-10 was also excluded because its humeral length is disproportionately long for the body size.

Data source.—The Xingyi Fauna is well known for abundant specimens of the pachypleurosaur *Keichousaurus hui* (Jiang et al. 2009), the first Triassic marine reptile taxon described from China (Young 1958). It was found in the Zhubangpo Member of the Falang Formation (latest Ladinian, Middle Triassic) in a wide area ranging from Dingxiao and Wusha (Xingyi, Guizhou Province) to Luoping and Fuyuan (Yunnan Province) (Li 2006). This study includes 107 specimens, all from the Middle Triassic of Xingyi City, Guizhou, China. Seventy specimens are from Cheng et al. (2009) and accessioned in NMNS, 11 from Lin and Rieppel (1998) and stored in BHNM, GPM, and IVPP, and four from Zhang (2011) and housed in GMPKU. We added 22 new specimens, including nine in the collection of GMPKU, and 13 left in situ at our excavation site in Xingyi; these latter specimens belong to WS.

**Physical measurements**.—All measurements were made with a pair of digital calipers with an accuracy of 0.01 mm, and a microscope was used for greater accuracy with small parts of the body (vertebrae, girdle bones, limbs, and skull). When the length of a body part exceeded the range of the calipers (150 mm), a ruler was used, and measurements then recorded to the nearest 0.1 mm. Some parts, such as the neck, trunk, and tail are not always preserved in a straight line, and in such cases, a cotton thread was aligned with the body parts and measured by the ruler later. Fourteen features were measured (see Table 1 in Supplementary Online Material available at http://app.pan.pl/SOM/app60-Xue_etal_SOM.pdf). The orientation of exposure, i.e., dorsal or ventral, was noted. The mean of measurements from both sides of the body was computed and used in the analyses. After averaging, measurements were logarithmically transformed to base 10.

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tem. Linear regression is based on Standardized Major Axis (SMA) in double logarithmic space, which was computed using R 3.0.0.

Results

Gender identification.—Our gender determinations (SOM: Table 1: gender 1) verify those given by Cheng et al. (2009) for those specimens included in their study. The LDA output of training data (Fig. 2, SOM: Table 2: gender 2) shows a separation between sexes without any overlap. This confirmed gender 2 was used as the working gender in all subsequent analyses.

Factors behind morphological disparity.—The result of EFA (Table 1) shows that three factors are sufficient to explain the covariance structure among all the measurements, with a p-value of 0.286 (the factors would be insignificant if the p-value was less than 0.05). Factor 1 largely reflects neck length, s-v length and standard length, which represent body length. Eigenvalues show that this is by far the most dominant factor. Factor 2 mostly depends on limb length and maximum width (distal width) of the humerus relative to the rest of the body, which represents sexual dimorphism. Factor 3 represents widths of the limb relative to other dimensions, probably reflecting sexual dimorphism to some extent. A bivariate plot of Factor 2 versus Factor 1 (Fig. 3) shows that the two sexes are largely separated by Factor 2, but not Factor 1.

Dimorphism in allometry.—Our analysis indicates that, in addition to the skeletal ratios suggested before, the relative size of the femur to the rest of the body also exhibits sexual dimorphism in Keichousaurus hui; it is usually longer in sex y than in sex x for a given snout-vent length. Combining this feature with the three ratios used in the initial gender identification gives four skeletal ratios that are sexually dimorphic. Bivariate plots of the measurements related to these four ratios (Fig. 4) show that sexual dimorphism is already present at birth in all four. However, these four metrics vary in the degree to which they express the dimorphism. In three of the four features, namely humerus length

<table>
<thead>
<tr>
<th>Item</th>
<th>Factor 1</th>
<th>Factor 2</th>
<th>Factor 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neck length</td>
<td>0.80</td>
<td>0.53</td>
<td></td>
</tr>
<tr>
<td>Tail length</td>
<td>0.71</td>
<td>0.48</td>
<td></td>
</tr>
<tr>
<td>Snout-vent length</td>
<td>0.78</td>
<td>0.54</td>
<td>0.31</td>
</tr>
<tr>
<td>Standard length</td>
<td>0.76</td>
<td>0.55</td>
<td>0.32</td>
</tr>
<tr>
<td>Humerus length</td>
<td>0.61</td>
<td>0.73</td>
<td></td>
</tr>
<tr>
<td>Femur length</td>
<td>0.65</td>
<td>0.68</td>
<td>0.32</td>
</tr>
<tr>
<td>Radius length</td>
<td>0.59</td>
<td>0.76</td>
<td></td>
</tr>
<tr>
<td>Ulna length</td>
<td>0.60</td>
<td>0.75</td>
<td></td>
</tr>
<tr>
<td>Tibia length</td>
<td>0.66</td>
<td>0.66</td>
<td>0.30</td>
</tr>
<tr>
<td>Fibula length</td>
<td>0.65</td>
<td>0.69</td>
<td></td>
</tr>
<tr>
<td>Humerus width at distal end</td>
<td>0.46</td>
<td>0.77</td>
<td>0.33</td>
</tr>
<tr>
<td>Humerus width at the middle of the shaft</td>
<td>0.67</td>
<td>0.52</td>
<td>0.45</td>
</tr>
<tr>
<td>Femur width at distal end</td>
<td>0.46</td>
<td>0.68</td>
<td>0.45</td>
</tr>
<tr>
<td>Femur width at the middle of the shaft</td>
<td>0.61</td>
<td>0.55</td>
<td>0.49</td>
</tr>
<tr>
<td>SS loadings</td>
<td>5.94</td>
<td>5.80</td>
<td>1.60</td>
</tr>
<tr>
<td>Proportion Variance</td>
<td>0.42</td>
<td>0.41</td>
<td>0.11</td>
</tr>
<tr>
<td>Cumulative Variance</td>
<td>0.42</td>
<td>0.84</td>
<td>0.95</td>
</tr>
</tbody>
</table>

Fig. 2. Histogram of the linear discriminant analysis scores for the specimens, showing clear separation between males and females. See Table 2 for the data.

Fig. 3. Bivariate plot of Factor 1 against Factor 2, showing clear separation between males and females. 95% confidence ellipses are indicated. See Table 3 for details.
relative to SVL (Fig. 4A), femur length relative to SVL (Fig. 4B), and humerus length relative to femur length (Fig. 4C), dimorphism is subtle at small body size. The onset of sexual dimorphism, as judged by the intersection between male and female regression lines, occurs at SVL of 27.7 mm for humerus length, and 29.7 mm for femur length. These values are smaller than 50 mm, which is the mean predicted birth size for *K. hui* (Cheng et al. 2009). In contrast, the distal expansion of the humerus is already pronounced even at very small size (Fig. 4D).

An F-test from the ANCOVA revealed that the lengths of both humerus and femur were significantly affected by gender, was and were not results of random fluctuations. The Pr(>F) value for the humerus is < 2e-16, and for the femur is 7.82e-10. The F-test confirmed our suggestion that the femur has a value in gender identification.

**Discussion**

**Gender identification.**—The two morphotypes identified here were considered to be two sexes, not two species for three reasons. First, the specimens all come from the same member of one geological formation, sharing the same texture of surrounding rock, suggesting that they shared similar temporal and geographic ranges. Second, they all have the diagnostic characters of *Keichousaurus hui* identified by previous workers (Lin and Rieppel 1998), sharing similar skull...
morphology (Holmes et al. 2008), vertebral and rib counts, and body size. Third, the proportion of males among the adults (24 out of 53) is statistically indistinguishable from 0.5: the 95% confidence interval for the true proportion behind this observed proportion is between 0.32 and 0.59 based on a two-tailed chi-square test with Yates’ continuity correction (df = 1, chi-squared = 0.65). Adults were identified based on the threshold SVL values suggested by Cheng et al. (2009), namely 126 mm for males and 122 mm for females. Whereas there is no necessity for the male proportion to be 0.5, the confidence interval suggests that both genders were present in similar proportions.

It should be noted that the male proportion among subadults is too small (4 out of 21). The same chi-square test suggests that the proportion is statistically smaller than 0.5, with the 95% interval of the male proportion lying between 0.03 and 0.24. This probably means that we are misidentifying some subadult males as females, and further information is required to resolve this problem.

Factors behind morphological disparity.—As reported earlier, EFA indicated that two factors, Factor 2 and probably Factor 3, reflected sexual dimorphism, whereas Factor 1 largely depended on body size. This is consistent with our expectation that age and gender are the most basic controlling factors in morphogenesis, assuming that body size continuously increased with age. It is important to note that the result of EFA depends on the choice of function and rotation to be used. Different functions or rotations may result in slightly different combinations of factors. Therefore, we can only infer, but not determine, from the result that age and gender are two major factors, or have most significant connection with two major factors behind the covariance structure of those measurements.

Dimorphism in allometry.—Of the four sexually dimorphic skeletal ratios mentioned earlier, distal expansion of the humerus is the most evident feature that is recognized across body size. All subadult males were identified based on this ratio alone. Given that this feature is already pronounced in the smallest male in the data, it most likely became established during an embryonic stage. The other three features are not very remarkable at birth size, and then develop over time until they eventually exceed the threshold values suggested by Cheng et al. (2009). However, it is important to recognize that males and females grew along different trajectories even before these features are sufficiently pronounced to exceed the thresholds. Given Fig. 4A–C, these different growth trajectories must have been initiated late in embryonic growth.

Conclusions

Ontogeny must be understood in order to give a complete description of a species. Allometric growth and sexual dimorphism are known in many species, including Keichousaurus hui, a small marine reptile of the Middle Triassic. Sexual dimorphism in K. hui is best expressed in relative lengths and widths of limb bones. One of the two morphotypes, with shorter and less stout limb bones, has been identified as the female based on specimens of gravid individuals (Cheng et al. 2004). In addition to three skeletal ratios listed by Cheng et al. (2009), we identified the relative length of femur to snout-vent length as an important marker of sexual dimorphism.

Our analysis showed that the two genders of Keichousaurus hui were unambiguously identified through a Linear Discriminant Analysis of skeletal measurements. However, further refinement of the method is probably necessary because the proportion of males among juveniles and subadults is too low. We also found, through Exploratory Factor Analysis, that body size and gender are the most significant factors behind the observed morphological disparity in this species. Most of the sexually dimorphic features are not very pronounced at birth size. However, analyses of allometry suggest that different growth trajectories between males and females are already set by that body size. Distal expansion of the humerus is an exceptional feature in that it is already pronounced in males at birth size. Identification of sexual dimorphism in extinct animals is crucial to our understanding of population structure and dynamics, diversity, and evolutionary history (Barden and Maidment 2011). Our study helps cultivate the way to improve such studies.

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