Combined paleohistological and isotopic inferences of thermometabolism in extinct Neosuchia, using *Goniopholis* and *Dyrosaurus* (Pseudosuchia: Crocodylomorpha) as case studies

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**Abstract.**—The evolution of thermometabolism in pseudosuchians (Late Triassic to the present) remains a partly unsolved issue: extant taxa (crocodilians) are ectothermic, but the clade was inferred ancestrally endothermic. Here we inferred the thermometabolic regime of two neosuchian groups, Goniopholididae (Early Jurassic to Late Cretaceous) and Dyrosauridae (middle Cretaceous to late Eocene), close relatives of extant crocodilians, in order to elucidate the evolutionary pattern across Metasuchia (Early Jurassic to the present), a clade comprising Neosuchia (Early Jurassic to the present) and Notosuchia (Middle Jurassic until the late Miocene). We propose a new integrative approach combining geochemical analyses to infer body temperature from the stable oxygen isotope composition of tooth phosphate and paleohistology and phylogenetic comparative methods to infer resting metabolic rates and red blood cell dimensions.†*Dyrosaurus* and †*Goniopholis* share with extant crocodilians similar lifestyles, body forms, bone tissue organization, body temperatures, metabolic rates, and red blood cell dimensions. Consistently, we infer ectothermy for †*Dyrosaurus* and †*Goniopholis* with the parsimonious implication of neosuchians and metasuchians being primitively ectothermic.

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Accepted: 23 September 2021

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**Introduction**

Thermometabolism is a key feature in the evolution of vertebrates. Two thermogenetic regimes have been identified: endothermy and ectothermy. The first regime involves the production of metabolic heat through nonshivering thermogenesis (Clarke and Pörtner 2010; Rowland et al. 2015), whereas in the second regime, the heat is mainly captured from the environment by behavioral adjustments (Clarke and Pörtner 2010; Rowland et al. 2015). Endotherms have a more elevated body temperature and a higher metabolic rate (i.e., the rate of oxygen consumption measured in ml(O2) h⁻¹) than ectotherms (Clarke and Pörtner 2010).

The origin of mammalian and avian endothermy (the only extant endothermic vertebrates) is a central question in evolutionary biology, because higher metabolic rates allow an extension of potential ecological niches, a higher growth rate, and better stamina for active behaviors (McNab 1978; Bennett and...
Ruben 1979; Walter and Seebacher 2009). Its presence in both non-avian dinosaurs and non-mammalian synapsids has been extensively investigated using many proxies (e.g., de Ricqlès 1974; Ruben 1995; Amiot et al. 2006; Cubo et al. 2012; Ruben et al. 2012; Legendre et al. 2016; Rey et al. 2017). However, in recent years, another group has been drawing the attention of paleobiologists: Pseudosuchia. Seymour et al. (2004) analyzed the pulmonary and cardiovascular systems of extant crocodilians and suggested that archosaurs were ancestrally/primitively endothermic. These features include the crocodilian four-chambered heart (Seymour et al. 2004) and the complex arrangement of pulmonary bronchia (Seymour et al. 2004; Farmer and Sanders 2010; Sanders and Farmer 2012; Farmer 2015), similar to those observed in birds. Farmer and Sanders (2010) showed that, more than the general anatomy of the pulmonary system, birds and crocodiles share a unidirectional air flow during respiration, a feature allowing birds to achieve high metabolic rates.

However, despite all these features, crocodilians (as ectotherms) do not display a high metabolic rate (Seymour et al. 2013). Seymour et al. (2004) suggested that the presence of these features in crocodilians is a legacy from their archosaurian endothermic ancestor. It would be congruent with other particularities, such as the presence of fibrolamellar bone (Padian et al. 2004; Tumarkin-Deratzian 2007) and the fast evolution of their mitochondrial genome (Janke and Arnason 1997; Janke et al. 2001), potentially related to high metabolic rate (Seymour et al. 2004).

Using quantitative bone histology, Legendre et al. (2016) concluded that the last common ancestor of all archosaurs possessed a high metabolic rate and was endothermic. Therefore, one can ask when (both temporally and phylogenetically) pseudosuchians lost their endothermy. Seymour et al. (2004) proposed a reversion sometime during the Jurassic (201–145 Ma), concomitant with the spread of ambush-type predation and semiaquatic lifestyle of numerous crocodylomorphs, features strongly associated with the particularities of extant crocodilians’ cardiovascular systems. While Legendre et al. (2016) concluded that archosaurs were ancestrally endothermic, their sample did not allow testing Seymour et al.’s (2004) hypothesis about a reversion to ectothermy in crocodylomorphs.

Legendre et al. (2016) included aetosaurs and rauisuchians in their sample, and their results suggest that all non-crocodylomorph pseudo- suchians were endothermic. Since then, new studies brought more data to this subject. Hence, Séon et al. (2020) used isotopic geochemistry to infer the body temperature ($T_b$) of thalattosuchians. They inferred ectothermy in Teleosauridae, and a more complex picture in Metriorhynchidae, interpreted as being heterothermic endotherms. Moreover, using quantitative histology, Cubo et al. (2020) suggested a common ectothermic ancestor for all notosuchians. Thus, assuming that archosaurs are primitively endotherms, these findings raise two parsimonious possibilities: either a single reversion to ectothermy at the node Crocodylomorpha (or before), and then a reoccurrence of endothermy in metriorhynchids, or converging losses of endothermy in Teleosauridae, Notosuchia, and Eusuchia. Therefore, the thermometabolic status of Metasuchia must be elucidated. While we are aware of the status of extant ectothermic crocodilians and notosuchians, neither Goniopholididae nor Dyrosauridae has been analyzed to date. The aim of this study is to infer the thermometabolic condition of both groups using for the first time two independent proxies in an integrative approach: bone paleohistology and stable isotope geochemistry. Different proxies have been proposed to infer the thermometabolism of extinct species. Some authors looked for the presence of morphological features, such as fur or feathers and respiratory turbinates, as indicators of the presence of homeothermy, which is strongly associated with endothermy (e.g., Ruben 1995; Ji et al. 2006; Ruben et al. 2012; Zhou et al. 2013). However, these features are only indirectly linked to endothermy, and the potential causal links are still being debated. Other studies proposed the modeling of the thermic exchange with the environment based on the 3D reconstruction of extinct organisms (e.g., Florides et al. 2001). Another possibility is the study of cardiovascular physiology, as several parameters are associated with the metabolic rate (for a review, see
Seymour 2013, 2016). Bone histology was extensively used in this area, both qualitatively and quantitatively, for its strong connection with metabolic rate (e.g., de Ricqlès 1974; Padian et al. 2004; Montes et al. 2007; Olivier et al. 2017; Fleischle et al. 2018; Cubo and Jalil 2019; Faure-Brac and Cubo 2020). Finally, stable isotope geochemistry was extensively used to infer the body temperature of extinct species (Amiot et al. 2006; Bernard et al. 2010; Rey et al. 2017; Séon et al. 2020).

Bone Histology

Amprino (1947) proposed that the structure of bone tissue records the bone growth rate (BGR). This finding was corroborated by Montes et al. (2010) in a phylogenetic context. In parallel, a link between BGR and resting metabolic rate (RMR, a standardized metabolic rate taking account of the effect of body mass) was shown by Montes et al. (2007). It is worth noting that the first relationship had been tested experimentally (Starck and Chinsamy 2002), whereas the relationship between RMR and BGR is correlational. A test of a causal relationship is lacking. Although correlational, we can use the quoted relationship to infer the RMR of extinct species using bone structure in phylogenetic comparative approaches.

Two mechanisms of osteogenesis that produce different bone structures have been described. The first, static osteogenesis (SO), is related to high BGR. SO quickly produces highly vascularized bone tissues composed of woven bone (isotropic fibers) and globular osteocyte lacunae displaying radiating canaliculi (Marotti 2010; Prondvai et al. 2014; Stein and Prondvai 2014; Cubo et al. 2017). The second, dynamic osteogenesis (DO), is associated with low BGR. DO produces nearly to completely avascular bones composed of parallel fibered bone that appears either as anisotropic tissue containing elongated lacunae when fibers run perpendicular to the direction of transmission of cross-polarized light or as isotropic and containing pinhead-shaped lacunae when fibers run parallel to the direction of transmission of cross-polarized light (Marotti 2010; Prondvai et al. 2014; Stein and Prondvai 2014; Cubo et al. 2017). To sustain rapid growth, SO requires higher amounts of energy than does DO, that is, a higher metabolic rate (Montes et al. 2007). However, identifying SO structures is not a sufficient criterion to suggest an endothermic condition, as exceptions occur, notably in extant crocodilians (Padian et al. 2004; Tumarkin-Deratzian 2007).

As an alternative, Legendre et al. (2016) proposed to use a phylogenetic comparative method, phylogenetic eigenvector maps (PEM; Guénard et al. 2013) to infer RMR values for extinct species using histological variables and phylogenetic relationships of a sample of extant vertebrates as explanatory variables. This method of quantitative histology was extensively used in various groups of vertebrates and provided promising results (Legendre et al. 2016; Olivier et al. 2017; Fleischle et al. 2018; Cubo and Jalil 2019; Cubo et al. 2020; Faure-Brac and Cubo 2020). As endothermy necessitates high RMR to fuel heat production, inferring the latter seems an appropriate proxy to infer the thermometabolic regime of extinct taxa.

Recently, another approach using quantitative histology was proposed by Huttenlocker and Farmer (2017). They showed a high correlation between red blood cell (RBC) width (RBC_w) and area (RBC_a) with the size of the bone vascular cavities. This finding is consistent with the observation that the smallest RBCs are found in vertebrates with the highest energetic expenditure, that is, the highest metabolic rate (mammals and birds). Indeed, smaller RBCs are associated with faster O_2 kinetic uptake (Holland and Forster 1966), allowing higher metabolic rates. Huttenlocker and Farmer (2017) were able to infer RBC width and area for extinct species using quantitative histology, and their methodology was then applied by Cubo et al. (2020), using PEM, with success. The combined use of these proxies is valuable, as they produce independent inferences for the same variable, thermometabolism.

Isotopic Geochemistry: Basic Principles

Relationships between Vertebrate Body Water, Diet Water, and Ambient Water.—The oxygen isotope composition of phosphate in vertebrate apatite (δ^{18}O_p) depends on both the mineralization temperature of the skeleton and the oxygen...
isotope composition of its body water ($\delta^{18}$Obw) (Kolodny et al. 1983; Longinelli 1984; Luz et al. 1984). For all vertebrates, the $\delta^{18}$Obw value depends on the oxygen isotope composition of diet water ($\delta^{18}$Odw), which mainly includes drinking water and water-bearing solid food (e.g., leaves, herbs, roots, meat). The oxygen isotope composition of diet water is itself directly related to the oxygen isotope composition of ambient water ($\delta^{18}$Oaw); which means freshwater (river, lake) or seawater ($\delta^{18}$Osw) for aquatic vertebrates and meteoric waters ($\delta^{18}$Omw) for terrestrial vertebrates (Luz et al. 1984; D’Angela and Longinelli 1990; Kohn 1996; Langlois et al. 2003). Aquatic vertebrates such as fish have $\delta^{18}$Obw values similar to those of the water masses where they live. In the case of semiaquatic vertebrates, $\delta^{18}$Obw values are influenced by their aquatic environments but also by a combination of input and output oxygen fluxes. Input oxygen fluxes to the animal body are air through the lungs and associated water vapor, food, and drinking water. Output oxygen fluxes from the animal body are feces and urinary water, urea or uric acid, transcutaneous and exhaled water vapor (via respiration or, for some mammals like carnivores, via panting), and expired carbon dioxide (Fig. 1).

Considering the oxygen isotope fractionation that takes place during evapotranspiration (evaporation of body water) and the combustion of food by atmospheric oxygen that has a $\delta^{18}$O value much higher (+23‰) than those of other reservoirs ($-3‰ < \delta^{18}$Osw < $+2‰$; $-30‰ < \delta^{18}$Omw < $+2‰$), $\delta^{18}$Obw values commonly increase by a value between 0 and $+2‰$ for most known semiaquatic and air-breathing vertebrates (Barrick et al. 1999; Amiot et al. 2007). Such heavy oxygen isotope enrichment of the body water relative to the diet water may reach up to $+6‰$ for terrestrial vertebrates (Longinelli 1984; Wolf et al. 2013).

**Relationships between Vertebrate Body Temperature and Ambient Water.**—In the framework of this study, the calculation of crocodylomorph body temperature is performed according to the following protocol and assumptions:

- The enrichment in heavy oxygen isotope ($^{18}$O relative to $^{16}$O) of crocodylomorph body water relative to ambient water does not exceed $+2‰$ (Amiot et al. 2007).
- The generic equation, which relates the $\delta^{18}$Op to mineralization temperature $T$ and $\delta^{18}$Oaw, is expressed as follows according to Kolodny et al. (1983) as modified by Lécuyer et al. (2013):

$$T = 117.4 - 4.5(\delta^{18}O_p - \delta^{18}O_{aw})$$  \hspace{1cm} (1)

becoming equation (2)

$$T = 117.4 - 4.5(\delta^{18}O_p - \delta^{18}O_{bw})$$  \hspace{1cm} (2)
when transposed to the case of aquatic or semiaquatic vertebrates.

- The use of isotopic fractionation equations that relate $\delta^{18}O_p$ to $\delta^{18}O_{aw}$ in the case of extant mammals, theropods, and turtles.

  - For mammals (Amiot et al., 2004):
    \[
    \delta^{18}O_{aw} = 1.1128 \times (\delta^{18}O_p - 26.41) \tag{3}
    \]
  
  - For theropods (Amiot et al., 2015):
    \[
    \delta^{18}O_{aw} = 1.119 \times (\delta^{18}O_p - 24.222) \tag{4}
    \]
  
  - For turtles (Barrick et al. 1999, modified by Pouech et al. 2014):
    \[
    \delta^{18}O_{aw} = 0.994 \times (\delta^{18}O_p - 21.197) \tag{5}
    \]

- The oxygen isotope composition of ambient water $\delta^{18}O_{aw}$ (mainly derived from meteoric waters) is estimated by using fractionation equations applied to theropods, mammals, or turtles co-occurring with studied dyrosaurids and †Goniopholis.

- Finally, equation (2) is solved for body temperature, $T_b$, by using previously calculated $\delta^{18}O_{aw}$ (equations 3–5) with the addition of body water $^{18}O$-enrichment to obtain $\delta^{18}O_{bw}$ and measured $\delta^{18}O_p$ of Dyrosauridae and †Goniopholis.

**Graphic Representation of Calculations and Data for Discussion of the Thermophysiological Status.**—Calculations and data are finally highlighted by using a $\delta^{18}O_p$–$\delta^{18}O_{aw}$ bivariate plot that discriminates between the fields of ectothermic–poikilothermic and endothermic–homeothermic vertebrate taxa. Those fields are constructed on the basis of a nomogram that represents body isotherm values. If tested dyrosaurids and †Goniopholis had ecto-poikilothermic thermophysiology similar to those of extant crocodilians, their $T_b$ values would fall within a preferred range of 26°C–36°C (Markwick 1998). If they were endotherms, then their body temperature would most likely fall within the 36°C–40°C range, as in most modern endotherms. Thus, the distribution of data in the predefined fields of body temperatures will offer a basis for discussing the thermophysiological status of vertebrate taxa studied.

To infer the thermometabolic condition of Goniopholididae and Dyrosauridae, both bone histology and isotopic geochemistry were used. We describe the bone cross sections of two specimens of these groups and use them to infer RMR, RBC$_w$, and RBC$_a$ using quantitative histology. We use already published isotopic data for Goniopholididae and present here new isotopic data for Dyrosauridae, with the aim of estimating their $T_b$. Finally, we propose a putative state of their thermometabolism using all these parameters. The null hypothesis is that they were ectothermic organisms because of their phylogenetic proximity and ecological similarity with extant crocodilians.

**Materials and Methods**

**Material**

Quantitative Histology.—Two extinct species were studied: †Goniopholis simus Owen, 1879 from the Berriasian (Early Cretaceous) of Cherves-de-Cognac, France (Muséum national d’Histoire naturelle/Paris Museum of Natural History: MNHN CHE.02.046) and †Dyrosaurus sp. Pomel, 1894 from the Ypresian (Eocene) of North Africa. For all species, both extinct and extant, we studied the femur.

Their data were incorporated into two datasets comprising histological variables and the variables of interest (RMR or RBC width/area) of a set of extant species. Both datasets are from Cubo et al. (2020). The first one, used in RMR inferences, comprises 18 extant species. The second one, used in RBC inferences, comprises 14 extant species. The complete list of included species is given in the Supplementary Material and Methods.

Isotopic Geochemistry.—Depending on the amount of available material, we sampled enamel or a mixture of enamel–dentine of several teeth coming from Paleocene dyrosaurid crocodylomorphs, from the pantodont eutherian †Alcidedorbignya inopinata de Muizon & Marshall, 1992, both provided by C. de Muizon, and a shell bony plate from the Podocnemidid turtle †Roxochelys sp. Price, 1953 recovered from the paleontological site of Tiupampa (Bolivia).
The details of the samples are given in Supplementary Table 1.

Geochemical data of †Goniopholis sp. and its associated fauna from the Berriasian site of Cherves-de-Cognac (France) were already published by Pouech et al. (2014). A selection of relevant values including mammals, turtles and theropod dinosaurs was then reanalyzed in terms of †Goniopholis Tp.

Methods

Preparations of Sections.—Thin sections of †Goniopholis simus and †Dyrosaurus sp. were prepared at the closest possible level to the middle of the diaphysis. For each species, one cross section from the mid-shaft of a femur was prepared and consequently analyzed. They were mounted on a glass slide following the method described in Lamm (2013). These sections were deposited at the vertebrate hard tissues histological collection of the MNHN, where they are available upon request to the curator, with the respective access numbers MNHN-F-Histos-2751 (†Goniopholis) and MNHN-F-Histos-2752 (†Dyrosaurus). The histological terminology follows Francillon-Vieillot et al. (1990) with addenda and revision from Prondvai et al. (2014).

Cross sections were observed under a Nikon Eclipse E600 POL microscope, either in linearly polarized light (LPL) or in cross-polarized light (XPL) with a lambda wave plate, in order to determine the nature of bone tissue in each topologic region using patterns of isotropy/anisotropy (Bromage et al. 2003; Faure-Brac et al. 2019). Variations in isotropy allowed us to assess orientation and organization of collagen fibers. Anisotropic fibers, which appear blue/yellow under XPL with a lambda compensator, are highly organized and parallel thus and perpendicular to the direction of propagation of XPL. For isotropic fibers, which appear red under XPL with a lambda compensator, two possibilities exist: either fibers lie in parallel and are parallel to the direction of propagation of XPL (i.e., cut transversally) or woven bone (for a review, see Faure-Brac et al. 2019).

Histological Variables.—Pictures were taken at different magnifications and analyzed with the software ImageJ v. 1.8.0_172 (Rasband 1997) to quantify the different histological variables. Three were quantified: relative primary osteon area (RPOA) for RMR inferences (described as primary osteon density by Fleischle et al. 2018) and harmonic mean caliber (HMC) and minimal caliber (canmin) of vascular cavities for inferences of RBC width/area (Huttenlocker and Farmer 2017).

RPOA was quantified as described by Fleischle et al. (2018): an area is delimited in the deepest primary cortex, and the surface occupied by primary osteons is quantified (see Fig. 2A). To quantify this variable in the deep, primary cortex is necessary, because our sample of extant animals only contains juveniles: therefore, in extinct adult taxa, we have to analyze the bone tissue placed as close as possible to their initial growth stage. RPOA corresponds to the ratio between the quoted variables:

\[
RPOA = \frac{S_{\text{osteon}}}{S_{\text{total}}}
\]

where \(S_{\text{osteon}}\) is the surface occupied by primary osteons, and \(S_{\text{total}}\) is the surface of the studied area. If some secondary bone is present in the total area, its surface is deducted from the \(S_{\text{total}}\) before the calculation of RPOA.

HMC and canmin of vascular canals were quantified in the outer cortex, as our sample of extant animals is composed of adults only. In accordance with Huttenlocker and Farmer (2017), up to fifty canals were quantified in each section (see Fig. 2B). We delineated canals and fit the largest ellipse in them. Then, we measured the minimal axis. HMC was calculated using the following equation:

\[
HMC = \frac{n}{\sum_{i=1}^{n}(1/x_i)}
\]

where \(n\) is the number of measured vascular cavities, and \(x_i\) the value of the minimal axis for the \(i^{th}\) cavity. canmin is simply the smallest minimal axis in our sample. The dataset of extant species used to infer RMR is from Cubo et al. (2020), and the dataset used to infer RBC variables is from Huttenlocker and Farmer (2017). All values for the extinct species were quantified in this study.

Phylogeny.—Two phylogenies were used, one for each set of taxa. The phylogeny used to infer RMR is an amended tree taken from Cubo et al. (2020), and the one used to infer
RBC variables is amended from Huttenlocker and Farmer (2017). In both cases, extinct species were removed and replaced with †Dyrosaurus sp. and †Goniopholis simus. Their placement follows the consensual phylogeny presented by Puértolas-Pascual et al. (2020). As the phylogenetic comparative method necessitates branch lengths, we dated the trees using the minimal age of apparition of each node. Branch lengths reflect, then, the time between the apparition of two successive nodes, or between a node and a leaf. Age of a given node was computed as the minimal age of the oldest fossil included in it and was taken from the Paleobiology Database (https://paleobiodb.org, last accessed 28 June 2020).

Phylogenetic Eigenvector Maps.—A summary of the principal steps of the protocol is presented here. The full protocol is available in Guénard et al. (2013) and in the Supplementary Material and Methods. PEM operates in two steps: (1) the construction of predictive models and the selection of the better performing one and (2) the inference of extinct species’ values.
For each of the three analyses, a model is chosen among a set of models comprising (1) a model including the phylogeny and the values of the studied variable of extant species and (2) many models comprising the phylogeny, the values of the studied variable of extant species, and one histological variable. After the choice of the model through the use of a corrected Akaike’s information criteria (AICc), inferences of RMR values and RBC dimensions for extinct species are computed. We used the package MPSEM (Guénard et al. 2013), with the software R v. 3.5.1 (R Development Core Team 2008). The script, data, and phylogenies used are given in the Supplementary Files.

Isotopic Geochemistry.—Chemical preparation and measure of the oxygen isotope composition of tooth and bone apatite phosphate were realized following the protocol published in Lécuyer et al. (1993) and detailed in the Supplementary Material and Methods. Phosphate from tooth enamel and bone was isolated in the form of silver phosphate crystals that were pyrolyzed at 1450°C using an elemental analyzer and then analyzed with an isotopic ratio mass spectrometer. The measurements were calibrated by performing a two-point calibration using the international reference materials NBS120c ($\delta^{18}$O$_{VSMOW} = 21.7\%$; Lécuyer et al. 1993) and NBS127 (BaSO$_4$, $\delta^{18}$O$_{VSMOW} = 9.34\%$; Gonfiantini et al. 1995). Isotopic compositions are quoted in the standard notation relative to VSMOW. Silver phosphate precipitated from standard NBS120c (natural Miocene phosphorite from Florida) was repeatedly analyzed ($\delta^{18}$O = 21.76 ± 0.12\%; $n = 20$) along with the silver phosphate samples derived from the vertebrate remains. Chemical extraction, analyses, and measurements were performed at the Laboratoire de Géologie de Lyon–Terre, Planète, Environnement.

**Results**

Histological Description

†Goniopholis simus.—The bone is partly fractured and invaded by fungi, making observations difficult to interpret in some places, especially in the posterior and dorsal areas (Fig. 3A). Fractured regions are not problematic: borders coincide most of the time, suggesting only a displacement of limited parts of the bone without loss of material. Regions invaded by fungi are especially problematic, as the fungi hide histological features of interest, such as the fibers’ organization. The majority of the section, however, displays a good preservation, allowing us to infer an interpretation of the paleobiology of †Goniopholis.

The section is nearly circular, displaying a large medullary cavity, free of spongy bone. The bone is slightly thicker in postero-dorsal areas, leading to a slightly off-center medulla (Fig. 3A). We observed a poor vascularization, predominantly concentrated in the deep layers of the cortex, associated with primary small-sized primary osteons. The fibers’ orientation is predominantly longitudinal (i.e., parallel to the main axis of the bone). The following histological description is based on observation of the anterior region (Fig. 3A, black square, B, E). Osteons appear associated in disorganized bundles in the deep cortex (Fig. 3B, layer A) but form circular rows closer to the periosteum (Fig. 3B, layers C$_1$–C$_4$). Except for a small part in the postero-dorsal region exhibiting some remodeling (Fig. 3F), tissue is mostly primary. The specimen is an adult, as suggested by the presence of an avascular outer circumferential layer, or external fundamental system (EFS; Fig. 3B, layer E).

This cross section is composed of alternating layers formed at a high growth rate (containing a scaffold of SO bone and primary osteons filled with DO bone: layers A and C$_1$–C$_4$ in Fig. 3B) and layers of DO bone tissue formed at low growth rate (Fig. 3B, layers B$_1$–B$_4$, D, E). The thick layer A is composed of a scaffold of SO bone containing primary osteons (Fig. 3B, layer A). Layers C$_1$ to C$_4$ have the same structure but are thinner than layer A (Fig. 3B, layers C$_1$–C$_4$). They are perfectly recognizable by the presence in the scaffold of SO osteocyte lacunae (globular, with radiating canaliculi) (Fig. 3C, black arrows) and isotropic tissue. In strong contrast, layers B$_3$ to B$_4$ display DO features (Fig. 3B, layers B$_3$–B$_4$), showing alternatively the two types of lacunae and fiber orientation (Fig. 3D, white and gray arrows). It can be explained by a plywood structure, that is, a succession of thin layers of lamellar bone having orthogonal orientations, visible under XPL.
These features suggest a first phase of rapid growth corresponding to the deep cortex (Fig. 3B, layer A). The outer part of the cortex, from the end of the deep cortex to the EFS, displays an alternation of periods of slow BGR (Fig. 3B, layers B1–B4), shown by the organized tissues, and periods of faster BGR, shown by the layers of osteons in circular rows (Fig. 3B, layers C1–C4). This organization, the lamellar-zonal, is typical of extant crocodilians (Padian et al. 2004; Tumarkin-Deratzian et al. 2007).

In addition to the primary bone, there are two kinds of secondary bone occurrences. The first one is present on the margin of the medullary cavity: endosteal bone. It consists of highly organized bone, completely avascular, exhibiting a strong anisotropy (Fig. 3F, white bracket). The second kind of secondary bone corresponds to the presence of secondary osteons (Fig. 3F, white arrowhead). However, remodeling is scarce, suggesting a young adult.

This pattern of alternating layers of SO and DO bone is observed everywhere in the section and is relatively homogenous, with some notable exceptions. The anterior area displays more bands of high growth rate than other areas, suggesting more active growth than in other areas. It is associated with the presence of Sharpey’s fibers, close to the periosteum (Fig. 3B, layer E). These fibers reveal the attachment of a soft tissue, muscles, or ligaments.

†Goniopholis presents a bone histology similar to that observed in extant crocodilians, with a lamellar-zonal histological organization. This animal was an adult according to the presence of the EFS but seems to be relatively young, as remodeling is still scarce. According to these features, it probably possessed a metabolic rate similar to that of extant crocodilians. Despite the fractures and fungal degradation, the section displays enough primary bone layers to allow a good quantification of variables and hence for performing the PEM analysis.

†Dyrosaurus sp.—The cross section of †Dyrosaurus is well preserved (Fig. 4A). There are some fractures, and we can identify invasion by fungi, but they are relatively scarce and do not impact the analysis of the section. The section is also nearly circular with a smaller medullary cavity compared with †Goniopholis; it is also free of bone trabeculae. Vascularization is dense but consists essentially of large secondary osteons. Primary osteons are scarce, but still present, and much smaller than the secondary ones. The vascularization is longitudinal in majority, as for †Goniopholis. The presence of an EFS suggests that this organism was an adult, and probably aged. Harvesian bone constitutes a large part of the section, and more than 15 lines of arrested growth (LAGs; Fig. 4D, white arrowheads, and visible on Fig. 3B, from layer B1 to E) are present in the outer half of the cortex. Therefore, tissue is essentially secondary in the deep cortex, which means that the recording of the first stages of life has been lost.

We observed two major types of primary bone. On one hand, the great majority of the remnant primary bone is constituted of DO bone: poorly vascularized, if not completely avascular (Fig. 4B, layers B1–B3), strongly anisotropic, and associated with elongated osteocyte lacunae (Fig. 4D, white arrows). Contrary to †Goniopholis, we cannot easily find an isotropic tissue with pinhead lacunae, suggesting there is no change in the direction of fibers.

On the other hand, we can find thin bands of circular rows of primary osteons as in †Goniopholis. These osteons are associated with isotropic fibers and globular lacunae, typical of SO bone (Fig. 4D, black arrows). As a large part of the bone is remodeled, it is difficult to
establish the exact number of these rows. It seems we can find at least four of them (Fig. 4B, layers C₁–C₄). Approaching the EFS, the rows become thinner. Moreover, on the outer half of the cortex, numerous LAGs are present. They are especially abundant and close to each other near the EFS. These features suggest a continuous growth, but steadily decreasing in magnitude and becoming negligible by the end of the specimen’s life.

Secondary bone is particularly present in this section. It consists of endosteal bone (Fig. 4C,...
TABLE 1. Results of quantification and phylogenetic eigenvector maps (PEM) inferences for both extinct studied species. n (ost) is the number of quantified osteones, n(can) the number of quantified vascular canals. HMC, harmonic mean caliber; RBC, red blood cell; RMR, resting metabolic rate; RPOA, relative primary osteon area.

| Species          | Histological variables | RMR (ml(O2) h⁻¹ g⁻⁰.⁶⁷) | RBC width (µm) | RBC area (µm²) |
|------------------|------------------------|---------------------------|----------------|----------------|
| †Dyrosaurus sp.  | n (ost) 85 RPOA 0.077   | 11.85 ± 1.75              | 165.94 ± 25.68 |
| °Goniopholis sinu.| n (can) 47 canmin 14.9  | 8.42 ± 1.17               | 132.35 ± 18.55 |
| °Goniopholis sinu. | canmin 21.5 HMC 0.144   |                           |                |
| °Goniopholis sinu. | canmin 20.5 HMC 0.139   |                           |                |
| °Goniopholis sinu. | canmin 10.3 HMC 0.130   |                           |                |

The bone is relatively homogenous, but it is tricky to identify differences in the growth pattern, as the secondary bone invaded a large part of the primary growth, especially the deeper half cortex. As for °Goniopholis, we observed a typical lamellar-zonal bone as currently documented in extant crocodilians. The extension of secondary bone is more problematic than for †Goniopholis, but there is still enough primary bone for measuring the required variables and performing PEM inferences.

Quantitative Histology.—The quantified variables were measured using equations (6) and (7) and are presented in Table 1. Extinct taxa display values of RPOA similar to those observed in Crocodylus (†Dyrosaurus: 0.077; °Goniopholis: 0.059; Crocodylus: 0.052). The same is found for both canmin (†Dyrosaurus: 21.5 µm; °Goniopholis: 20.5 µm; Alligator: 22 µm) and HMC (†Dyrosaurus: 14.9 µm; °Goniopholis: 10.3 µm; Alligator: 13.2 µm).

The AICc and $R^2$ values for each model and analysis are presented in Table 2. For all analyses, a model including a histological variable was chosen. For both RBC analyses, a model including the canmin as explanatory variable was chosen (RBC_w, $R^2 = 0.828$, AICc = 49.749; RBC_w, $R^2 = 0.981$, AICc = 128.285). It was unquestionably better than others for the RBC_a analysis and was preferred to the model including HMC ($R^2 = 0.843$, AICc = 54.455) because of its better AICc score and similar $R^2$ coefficients for the RBC_w analysis. However, for RMR analysis, model outputs with and without RPOA were similar. Although the model including the phylogeny exclusively is slightly better from a statistical point of view ($R^2 = 0.979$, AICc = 29.912), we chose to use a model including RPOA as an explanatory variable ($R^2 = 0.972$, AICc = 30.052), as it is more relevant from a biological point of view.

Using these models, we were able to infer values of RMR, RBC_w, and RBC_a for both extinct taxa. These values are presented in Table 1 and Figure 5 (see Supplementary Fig. 1 for a version with cross-values). Shapiro-Wilk tests were performed on residuals for all models. A log transformation was necessary for the model using RPOA for RMR inference ($p = 0.0374$ before transformation; $p = 0.8361$ after transformation). We used natural
logarithm for RMR and natural logarithm +1 for RPOA, as it contains null values. It was not necessary to transform the data of the two other analyses, as results of Shapiro-Wilk tests were not significant ($p = 0.9577$ for RBC$_w$ analysis; $p = 0.6624$ for RBC$_a$ analysis).

As data for RMR inferences were log transformed, the confidence interval is asymmetric after de-transformation. We found very low values of RMR for both taxa (†Dyrosaurus = $[0.23; 0.14-0.36]$ ml(O$_2$)h$^{-1}$ g$^{-0.67}$; †Goniopholis = $[0.21; 0.14-0.30]$ ml(O$_2$) h$^{-1}$ g$^{-0.67}$), close to what we observed in Crocodylus (0.331 ml(O$_2$) h$^{-1}$ $g^{-0.67}$) and in Caiman (0.12 ml(O$_2$) h$^{-1}$ $g^{-0.67}$). These values and the corresponding confidence intervals are lower than the maximal known value for an endotherm (cf. Microcebus, RMR = 1.526 ml(O$_2$) h$^{-1}$ $g^{-0.67}$) and are close to the maximal known value for an ectotherm (cf. Crocodylus, RMR = 0.331 ml(O$_2$) h$^{-1}$ $g^{-0.67}$; Fig. 5A). Hence, we can conclude both †Dyrosaurus and †Goniopholis had an RMR close to their extant relatives, within the range of ectotherms.

A similar situation is observed for RBC$_w$ with values inferred for both taxa similar to those observed in extant relatives (Alligator = 159.4 μm$^2$; †Dyrosaurus = 165.94 ± 25.68 μm$^2$; †Goniopholis = 132.35 ± 18.55 μm$^2$) and superior to the maximum known value for endotherms (cf. Columba = 69.5 μm$^2$) and the minimal known value for ectotherms (cf. Varanus exanthematicus = 99.4 μm$^2$; Fig. 5C). For the RBC$_w$ inference, the situation is similar for †Dyrosaurus (RBC width = 11.85 ± 1.75 μm), but the confidence interval of †Goniopholis (RBC width = 8.42 ± 1.17 μm) includes the maximal known values of endotherms (cf. Neovison = 7.8 μm). Both their central values are still close to the Alligator value (RBC$_w$ = 10 μm; Fig. 5B). Thus we can conclude that both taxa displayed RBC$_a$ in the range of extant ectotherms and †Dyrosaurus had RBC$_w$ in the range of extant ectotherms too. However, considering that the confidence interval of the inference for †Goniopholis overlaps the values observed in ectotherms and endotherms, no conclusion can be outlined for this taxon using this model.

Isotopic Geochemistry

Tiupampa.—Oxygen isotope composition of phosphates from Tiupampa vertebrates are presented in Table 3, along with the estimated δ$^{18}$O$_{aw}$ values calculated by using equations (3) to (5). Mammals point to a source of drinking water with an average δ$^{18}$O$_{aw}$ value of −5.4 ± 0.4‰, and the turtle †Roxochelys to a less negative value of −2.1‰, indicating at least two distinct sources of drinking water. Using these δ$^{18}$O$_{aw}$, we inferred the possible $T_b$ of Dyrosauridae using equation (2), which is reported in Table 4. $T_b$ estimates using †Alcedorhynchia inopinata's δ$^{18}$O$_{aw}$ as water sources are too low for crocodylomorphs to be realistic (10°C–23°C; Table 4; Fig. 6A). However, $T_b$ estimates using †Roxochelys's δ$^{18}$O$_{aw}$ as water sources range from 25°C to 38°C and appear more coherent (Table 4; Fig. 6A). This range of body temperatures covers that of modern crocodilians and is compatible with an ecto-poikilothermic thermometabolism for dyrosaurids.

Cherves-de-Cognac.—Published isotopic values from Pouech et al. (2014) include those of †Goniopholis along with those of the theropod cf. †Nuthetes, the turtles †Pleurosternon and †Tretosternon, as well as the mammals †Pinheirondon and some indeterminate Spalacotheriidae and Dryolestidae (Table 3). Using equations (3) to (5), local water δ$^{18}$O$_{aw}$ values have been calculated and two major sources have been identified: the first one, calculated from both turtles and theropods, ranges from 1.0 ± 1.0‰ to 1.2 ± 0.3‰; and the second one, calculated from mammals, has an average value of −3.3 ± 1.3‰ (Table 4). $T_b$ estimates...
using mammals’ δ¹⁸Oaw values as water sources are too low for crocodylomorphs to be realistic (12°C–16°C). However, using turtles’ and theropods’ δ¹⁸Oaw values as water sources, Tₚ estimates, which range from 31°C to 36°C (Table 4; Fig. 6B), cover that of modern crocodilians and are compatible with an ecto-poikilothermic thermometabolism for †Goniopholis.

**Discussion**

**Histology**

Qualitative and quantitative histological analyses lead to the same conclusion: †Goniopholis and †Dyrosaurus possessed a thermometabolic regime similar to that of extant crocodilians. Their bone microstructure and histological organization depict a first burst of growth, early during the post-hatching ontogeny, followed by a succession of periods of relatively high and low BGR, as suggested by the lamellar-zonal organization. However, this is not definitive evidence to infer a low metabolic rate, as some endotherms display typical ectothermic bones (Reid 1984). It is worth considering that Legendre et al. (2016) inferred an endothermic metabolism for †Calyptosuchus (Aetosauria) and †Postosuchus (Rauisuchidae), based on their inferred RMR values, but their bone organization is lamellar-zonal too (de Ricqlès et al. 2003), similar to those observed in †Goniopholis and †Dyrosaurus. Therefore, qualitative histology seems insufficient to infer their metabolism.

The inference of RMR with quantitative histology supports the hypothesis of two ectothermic taxa. We preferred to use a model comprising a histological variable, as both models (phylogeny only and phylogeny combined with bone histology) are similar in terms of AICc and R² parameters. Moreover, in previous studies, RPOA was preferred to the model without it (Fleischle et al. 2018; Faure-Brac and Cubo 2020). Regardless, the choice of one model over other with equivalent scores does not matter, as it will only slightly modify the inferred variables. We inferred a low RMR value despite the fact that RPOA was quantified in layers displaying the highest BGR, hence associated to the highest RMR. The RBCa and RBCw inferences point in the same direction. For both variables, values inferred for †Dyrosaurus fall within the range of those

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**Table 3. Oxygen isotope composition of phosphate (δ¹⁸Op) from vertebrates of Tiupampa and isotopic values extracted from Pouech et al. (2014) for Cherves-de-Cognac are reported along with calculated oxygen isotope composition of their drinking water (δ¹⁸Ow) using phosphate water fractionation equations (3) to (5) (see text).**

| Taxa                              | δ¹⁸Op (%VSMOW) | δ¹⁸Ow (%VSMOW) | Source               |
|-----------------------------------|----------------|----------------|----------------------|
| Paleocene of Tiupampa (Bolivia)   |                |                |                      |
| Dyrosauridae indet.               | 20.4           | —              | This study           |
|                                   | 19.9           | —              | This study           |
|                                   | 17.5           | —              | This study           |
|                                   | 18.3           | —              | This study           |
| †Alcidedorbygna inopinata         | 18.5           | −5.8           | This study           |
|                                   | 18.7           | −5.6           | This study           |
|                                   | 19.2           | −5.0           | This study           |
|                                   | 19.3           | −4.9           | This study           |
| †Roxochelys sp.                   | 19.2           | −2.1           | This study           |
| Berriasian of Cherves-de-Cognac (France) |       |                |                      |
| †Goniopholis sp.                  | 22.1           | —              | Pouech et al. 2014   |
|                                   | 21.8           | —              | Pouech et al. 2014   |
|                                   | 21.3           | —              | Pouech et al. 2014   |
| †Pleurosternon sp.                | 21.6           | 0.3            | Pouech et al. 2014   |
| †Tretosternon sp.                 | 23.0           | 1.7            | Pouech et al. 2014   |
| Theropoda cf. †Nuthetes           | 22.5           | 1.0            | Pouech et al. 2014   |
|                                   | 23.0           | 1.5            | Pouech et al. 2014   |
|                                   | 22.6           | 1.1            | Pouech et al. 2014   |
| †Pinheirodon pygmaeus             | 22.1           | −1.8           | Pouech et al. 2014   |
| Spalacotheriidae                  | 20.1           | −4.0           | Pouech et al. 2014   |
| Dryolestidae                      | 20.2           | −3.9           | Pouech et al. 2014   |

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measured in extant ectotherms (characterized by the presence of large RBCs). A similar result is observed for †Goniopholis, but only for RBCa. Inferred values of RBCw for †Goniopholis are inconclusive, because the associated 95% confidence interval overlaps values measured in extant ectotherms and extant endotherms. The inference of RBCw is the least conclusive of our analyses, as the model is the less supported \( R^2 = 0.828 \) and should be viewed with more caution than the two others. As high values of RBCa and RBCw are causally linked to low metabolic rates (Holland and Forster 1966; Hutttenlocker and Farmer 2017; Cubo et al. 2020), our inferences of RBC dimensions are congruent with our RMR estimations. To achieve a higher metabolic rate and then endothermy, a higher amount of produced energy is required, which demands increased oxygen uptake to sustain the energetic production. The reduction of the size of RBCs is a powerful solution: smaller RBCs are more efficient supplying in oxygen (Snyder and Sheafor 1999; Soslau 2020).

All histological analyses draw the same general picture suggesting that both taxa probably shared a low RMR, typical of what we observed in their extant relatives. Therefore, on the basis of our data, we conclude that both †Dyrosaurus and †Goniopholis were ectothermic vertebrates.

Isotopic Geochemistry

The new isotopic data obtained from the Tiupampa fauna and a review of the Cherves-de-Cognac fauna data allowed us to estimate the \( T_b \) for both our taxa, using the available relationships linking \( T_b \) to \( \delta^{18}O_p \) and \( \delta^{18}O_{bw} \). Extant crocodiles are polyphiodonts, that is, their teeth are continually replaced during a large part of their life (Poole 1961). Therefore, we have access to a single brief record of \( T_b \) in the life of the specimen. As each tooth can be replaced up to 45 times in the lifetime of an extant crocodilian (Poole 1961), their formation records only a short moment of the animal’s life, but it is large enough to provide the entire range of thermal behavior of a typical semi-aquatic organism, such as extant crocodilians. †Goniopholis would be expected to follow that pattern if it was ectothermic. In contrast, dyrosaurids were marine in their great majority
(Wilberg et al. 2019). Although polyphiodonts, their living environment was much more thermally buffered because of their aquatic ecology. An ectothermic dyrosaurid should then have recorded an aquatic environment close to marine water; however, the estimated negative δ18O values indicate that dyrosaurids from Tiupampa most likely lived in a 18O-depleted freshwater environment such as an estuary or a river. Two dyrosaurid species have been documented so far, which are of similar geological age (the Maria Farinha Formation, Brazil): †Guarinisuchus munizi and †Hyposaurus derbianus, both known as marine species (Wilberg et al. 2019). Therefore, our studied dyrosaurids were either marine species living at the mouth of an estuary, being in this case similar to the species from the Maria Farinha Formation, or we sampled an unknown freshwater species.

Using water δ18Ow values estimated from coexisting turtles and from theropod dinosaurs at Cherves-de-Cognac, calculated dyrosaurid and †Goniopholis Tb values fall in the “preferred” temperature range of extant crocodilian body temperature (26°C–36°C). However, the calculated Tb values for both dyrosaurids and †Goniopholis are unrealistically low when using mammal-derived δ18Ow values. It is noteworthy that Pouech et al. (2014) considered it improbable that †Goniopholis shared the same environment as mammals, as the latter would be expected to live in forests and the former in an estuary-like environment, and similar reasoning could be proposed for the small pantodont mammals †Alcidedorbignya, which have been considered by de Muizon et al. (2015) to be a terrestrial animal with possible scansorial habits, compatible with a forest habitat. Therefore, the ambient waters ingested by mammals in both cases were most likely not the waters inhabited by dyrosaurids and †Goniopholis. Once again, we consider both Dyrosauridae and †Goniopholis as ectothermic using this proxy.

Integrative Approach

RMR and RBC dimensions inferred for †Dyrosaurus and †Goniopholis using quantitative histology fall in the range of variations measured in extant ectotherms. Moreover, oxygen isotopes of apatite phosphate suggest a range of body temperatures compatible with that of extant crocodilians. Despite the specific limits of both approaches, results converge, suggesting an ectothermic status for the two vertebrate taxa studied here, even though such interpretations must be made with caution.

Isotopic geochemistry only provides a rough estimate of Tb and depends on the proper estimation of drinking water δ18Ow value based on coexisting vertebrates’ δ18Ob values. Moreover, Tb is only a product of thermometabolism and not entirely driven by it. A high Tb is necessary but not sufficient in itself to infer endothermy. Gigantothermy could be a possibility to explain high body temperatures, but it still has to be proven that an endotherm-like Tb can be reached this way. Known extant cases of gigantothermy roughly attain ~30°C (Paladino et al. 1990). For extinct cases, it was theorized that large dinosaurs could achieve homoiothermy with a Tb of ~31°C (Seebacher et al. 1999).

Quantitative histology allows inferring metabolic rate using phylogenetic comparative methods. It possesses its known issues, as it cannot robustly detect ectothermy, because bone structure produced by an ectothermic animal can easily be formed by endothermic ones as well. Moreover, it cannot detect particular cases such as putative gigantothermy (high Tb but low RMR). In addition, we cannot be fully certain that a high RMR always indicates endothermy. As mentioned before, the link between these variables is correlational.

Conclusion

This study, along with those published by Cubo et al. (2020), Legendre et al. (2016), and...
Séon et al. (2020), explores the evolution of thermometabolism among the pseudosuchians and, more especially, the loss(es) of their ancestral endothermy (Seymour et al. 2004). A hypothesis suggesting a loss linked to the acquisition of a semiaquatic lifestyle and ambush predation was proposed (Seymour et al. 2004). However, hypothesizing that Pholidosauridae were ectotherms as Dyrosauridae (on the basis of their close phylogenetic relationship and shared similar lifestyles, morphologies, and predation type) would imply a unique loss of endothermy at the node Metasuchia as a parsimonious explanation (Fig. 7) or even earlier at the node Crocodyliformes or Crocodylomorpha. Therefore, ectothermy may be ancestral for all metasuchians. Wilberg et al. (2019) stated that a terrestrial lifestyle is ancestral among Crocodylomorpha and Metasuchia and that the acquisition of a

**FIGURE 7.** Summary of the distribution of thermometabolism across Archosauria. The phylogeny used is a handmade combination of both trees presented in Puértolas-Pascual et al. (2020) and Nesbitt (2011). Red indicates an inferred or known endothermic taxon. Blue indicates an inferred or known ectothermic taxon. Black indicates unknown status. Black surrounded by dotted colored lines indicates a putative endothermic/ectothermic status depending on the color used. Shapes were taken from phylopic.org.
A semiaquatic lifestyle and ambush predation is a specificity of Neosuchia. Seymour et al.’s (2004) hypothesis about an ancestral endothermic state for archosaurs is thus corroborated, but their hypothesis about the loss of endothermy linked to the acquisition of a semiaquatic lifestyle and ambush predation is rejected, because terrestrial Notosuchia are inferred to be ectotherms (Cubo et al. 2020) and Metasuchia are inferred to be ancestrally terrestrial. However, the ambiguous status of *Calsoyasuchus* could lead to a review of this interpretation if it considered to be amphibious (Wilberg et al. 2019: fig. 6).

Legendre et al. (2016) inferred endothermic-like RMR for †Postosuchus (Rauisuchidae) and †Calyptosuchus (Aetosauria), both external to the clade Crocodylomorpha. Séon et al. (2020) proposed ectothermic Teleosauridae and endothermic–heterothermic Metriorhynchidae, with a doubt concerning the ancestral status of thalattosuchians. The last missing pieces are the status of Protosuchidae and some stem species, like †Hsisosuchus, which should bring the final necessary data to constrain the reversion(s) of thermometabolic condition in Pseudosuchia (Fig. 7).

We suggest that combined bone paleohistology and stable isotope geochemistry should be used whenever possible to contrast inferences and produce more robust interpretations.

**Acknowledgments**

We thank L. Cavin, R. Allain, and V. de Bufrenil for providing access to the studied material. We thank S. Morel for the preparation of cross sections and D. Germain for the access to the hard tissue collection. The histology part of the study was designed by J.C.; the isotopic geochemistry part was designed by R.A. and C.L. All authors contributed equally in the analyses. M.G.F.-B. wrote the first version of the article, and all authors contributed equally to its improvement. We thank Roger Seymour, another anonymous reviewer, and our editor Matt Friedman for their constructive comments.

**Data Availability Statement**

Data available from the Dryad Digital Repository: [https://doi.org/10.5061/dryad.4xgxd259v](https://doi.org/10.5061/dryad.4xgxd259v).

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