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Medullary bone-like tissue in the mandibular symphyses of a pterosaur suggests non-reproductive significance

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Medullary bone is a special bone tissue forming on the endosteal surface of the medullary cavity in the bones of female birds prior to and during egg-laying to serve as a calcium reservoir for building the hard eggshell. It has also been identified in non-avian dinosaurs, where its presence is considered as a reliable indicator of a sexually mature female. Here, we reveal that multiple mandibular symphyses of the azhdarchid pterosaur *Bakonydraco galaczi* possess a special bone tissue that shows all microanatomical, histological, and developmental characteristics of medullary bone, despite its unusual location. Its frequent occurrence in the sample renders a pathologic origin unlikely. Our findings as well as the extremely thin-shelled eggs of pterosaurs suggest that this medullary bone-like tissue probably had a non-reproductive role in these animals. Although the non-reproductive significance and the anatomical location of this medullary bone-like tissue in *Bakonydraco* suggest independent evolutionary appearance from dinosaurian medullary bone, a common origin and later diverging function and physiological regulation is an equally viable phylogenetic hypothesis.

Medullary bone is a non-structural type of bone tissue that, under natural conditions, develops in the haematopoietic medullary cavities of bones of female birds induced by oestrogenic and androgenic hormones during the egg-laying cycle^{1,2}. Under experimental conditions, administration of these hormones to male birds also results in medullary bone formation^{3–5}. Growing from the endosteal layers inwards to the medullary cavity, and mostly being composed of woven bone⁶, medullary bone has no mechanical function but serves as a calcium storage that can be quickly mobilized during the calcification of the hard-shelled eggs of birds^{6–9}. According to the phases of the egg-laying cycle, medullary bone can be characterized by rapid formation in the period of maturing follicles, and subsequent rapid destruction during the calcification of the eggshell^{10–12}. This fast formation-resorption cycle of medullary bone corresponds well with its microanatomy as well as histology: (i) it has large surface areas due to its highly porous, vascularized nature; (ii) its trabeculae are composed of woven bone⁶; (iii) the number and activity level of osteoclasts is considerably higher in medullary bone than in cortical bone¹³. The composition of medullary bone is similar to that of cortical and cancellous trabecular bone; however, the proportional amount of components are different with medullary bone being more calcified, having higher apatite to collagen ratio, and containing more non-collagenous proteins, proteoglycans and carbohydrates in the matrix^{7,12,14,15}.

This bone tissue type was first described in pigeons¹⁶, and later in some other species of extant birds, among others in domestic fowl, Japanese quail, duck and ostrich^{3,10,11,17–21}. The amount, microanatomy and distribution of medullary bone can be different in different species of birds as well as in different phases of the reproductive cycle^{1,11,17,21,22}. Apart from the controversial results on mice forming medullary bone-like tissues in response to unnaturally high oestrogen doses^{23,24}, medullary bone has not been reported in any extant non-avian amniotes^{25,26}. Recently, with the discovery of medullary bone in different non-avian dinosaurs, the occurrence of medullary bone has proven to be phylogenetically more widespread and is considered as further evidence for the close relationship between birds and theropod dinosaurs^{21,27–29}. Apart from dinosaurs, there has been only one report on the presence of an extensive, endosteally derived bone structure in a single femur of the Early Cretaceous pterosaur *Pterodaustro* that was tentatively interpreted as medullary bone³⁰. Since it has been found in theropod^{21,27,29} as well as in ornithomimid^{27,28} dinosaurs but not in extant alligators^{31,32}, medullary bone has been suggested to have first appeared in the ornithomimid lineage of archosaurs after its divergence from crocodylians^{27,32}.

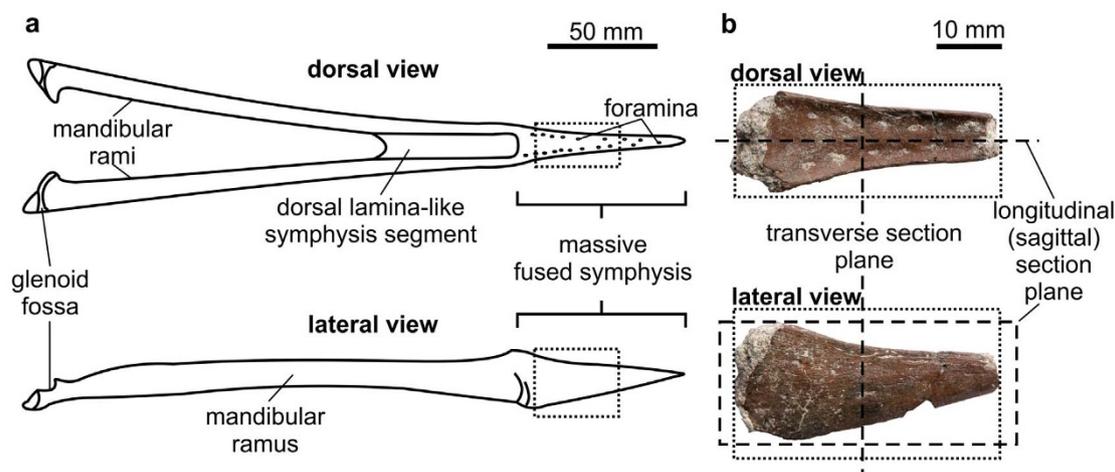


Figure 1 | Morphology and the histological sample location of a mandibular symphysis of *Bakonydraco galaczi*. (a) Silhouette drawing of the holotype lower jaw 2007.110.1 and (b) photograph of specimen 2007.111.1 (11) representing the preserved most robust part of the massive mandibular symphysis (stippled squares in (a)). Symphyses were sectioned in two cutting planes indicated by dashed lines and dashed square in (b).

Here we document the presence of a remarkable bone tissue in the mandibular symphyses of *Bakonydraco galaczi* (Fig. 1), an azhdarchid pterosaur from the Late Cretaceous (Santonian) of Hungary³³. Except for its unusual anatomical location, this tissue shows all microanatomical, histological, and inferred developmental characteristics of medullary bone as described in non-avian dinosaurs.

Results

In all ontogenetic stages present in our sample the mandibular symphyses are completely ossified without any histological sign of former fibrous connection or interdigitating arrangement of bone in the sutural region, as seen in the variety of syndesmoses characterizing the jaw symphyses of most lizards³⁴ and crocodiles³⁵. This refers to a developmentally early fusion of the mandibular rami resulting in a synostotic symphysis similar to most turtles and birds³⁵. Except for the ventrolateral and ventral symphyseal regions, an extensive erosion cavity system of highly variable spatial distribution, extent, and appearance characterizes all symphyses and reflects the very dynamically changing microanatomy and microstructure of these elements. Large cavities are always present in the sagittal plane (i.e. along the expected suture line). The numerous cavities become confluent posteriorly along the longitudinal axis of the symphyses. These microanatomical features are not compatible with the hypothesis that the irregular cavity system in the jaw symphysis of *Bakonydraco* solely housed the mandibular branch of the trigeminal nerve or such a distinct structure as Meckel's cartilage, the latter of which has a taxon-specific but an anatomically well-defined shape, size, and relative position in the mandible^{34,35}. Hence, this cavernous system is considered as medullary cavity subdivided by thick bony columns into recesses of variable sizes (Fig. 2 and Supplementary Figs S1 and S2) that, beside nerves and blood vessels, most likely contained bone marrow (or maybe air diverticula) as well. Extensive resorption and redeposition activity within and around these recesses is evident in all specimens; however, it is markedly more intensive in specimens identified as actively growing individuals than in the two skeletally mature specimens³⁶. The very intense remodelling results in the deposition of peculiar secondary bone tissues. Their histological appearance varies from highly porous and disorganized endosteal tissue to interwoven but highly compacted secondary bone. Transitional stages between these two extremes can be recognized among the specimens.

The microanatomical location, origin, morphology, and microstructure of the highly cancellous endosteal bone tissue found in

the histologically least mature specimens, 2007.111.1(15) (Supplementary Fig. 2), 2007.111.1(20) (Figs 2, 3), and 2010.74.16 (Fig. 4g–i), and in the histologically adult 2010.74.18 (Fig. 4j–l) correspond with the features characterizing medullary bone of dinosaurs, including birds^{21,27–29}. It is centripetally deposited on the endosteal surfaces of the cavities which are in most cases clearly separated from primary bone by a distinct resorption line and by endosteal lamellae of variable thickness (Figs 3c,e, 4h,j,k). It is highly vascularized by unusually wide canals and sinuses. The bony struts separated by these wide vascular spaces show low birefringence, and contain mostly spatially unorganized, irregularly shaped lacunae apparently derived from static osteogenesis (SO)^{37–39}. On the other hand, lacunae adjacent to the endosteal lamellae and those around the intact vascular canals exhibit dynamic osteogenesis-derived (DO)^{37–39} features (Figs 3b,d, and 4l). Thus, this loosely organized secondary medullary tissue is composed of a combination of woven and parallel-fibred bone with the former being the dominant component of the complex and supporting its fast growing nature. Based on the architectural and structural appearance of its bony struts, different phases of the bone deposition–resorption cycle can be inferred, sometimes even in the same section. In its fully developed and early resorption stage, the intact vascular canals are organized in a laminar to plexiform to irregular architecture (Figs 2d,e, and 3a–d, and Supplementary Fig. 2) and may show weak lamellar infilling (Fig. 3b). The beginning of the resorption phase is characterized by the appearance of small resorption pits (Howship's lacunae) on the surface of the bony spicules (Fig. 3d) due to osteoclastic activity. Thus, in this phase, vascular canals are widened by resorption resulting in irregular sinusoid channels. In a more advanced resorption state, the degradation of this secondary bone tissue extends through its sinusoid network even further, and results in larger erosion areas surrounded by scalloped bony surfaces (Fig. 3a,c,d), so that the original vascular architecture cannot be inferred anymore. The described architecture (Figs 2 and 3, and Supplementary Fig. S2) and histology (Figs 3 and 4) evidently show that this tissue is non-structural which clearly differs from both the dynamically changing structural network of load-bearing trabeculae and the irregularly arranged remnants of already resorbed cortical bone. The medullary bone-like tissue is most extensive in the posterior to middle section of 2007.111.1(20), but can also be traced to a lesser extent in the same region of 2010.74.16 and 2007.111.1(15), and in the anterior region of 2010.74.18.

The secondary tissues, less reminiscent of medullary bone in their overall morphology but still considerably perforated, can be found in

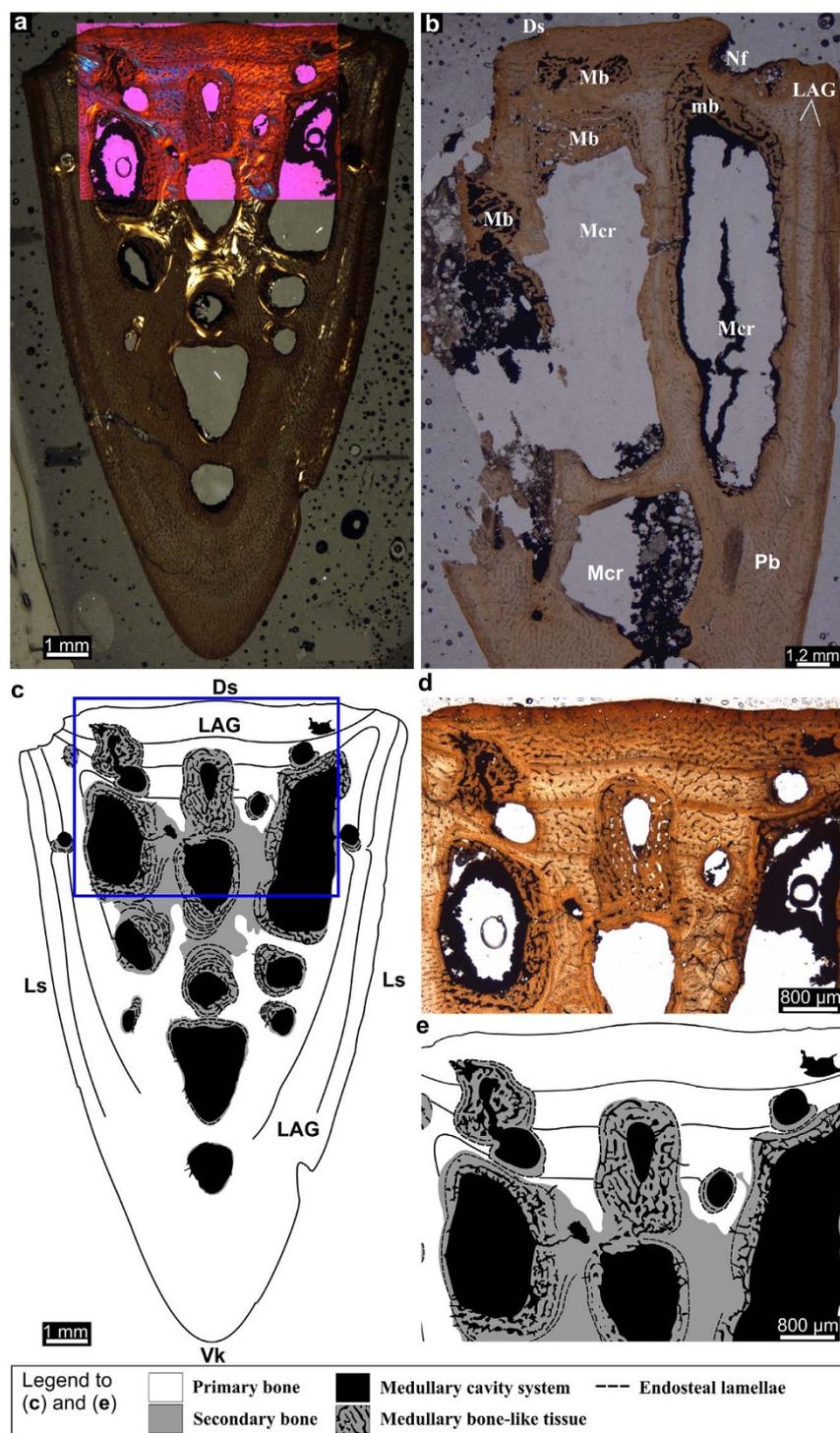


Figure 2 | Symphysis specimen 2007.111.1(20) in transverse section showing the most extensive amount of medullary bone-like tissue among all investigated symphyses. (a) Overview of a thin section under cross polarized light (inset aided by lambda wave plate) and (b) its posterior counterpart obtained from the same cut under single plane polarizers. The larger extent and merging of the recesses of the medullary cavity system is already evident in this slightly more posteriorly positioned symphyseal region. (c) Line drawing of (a) revealing the recesses of the medullary cavity system, the distribution of primary and secondary tissues, and the microanatomy, relative amount, and position of medullary bone-like tissue growing into the medullary cavity recesses (see also legend within the figure). (d) Close-up of the area marked by blue square in (c) under plane polarized light and (e) line drawing of the same area highlighting the microanatomy of the medullary bone-like tissue and the distribution of primary and secondary tissues as indicated by the legend. Abbreviations: Ds, dorsal symphysis surface; LAG, line of arrested growth; Ls, lateral symphysis surface; Mb, medullary bone-like tissue; Mcr, medullary cavity recess; Nf, nutritive foramen; Pb, primary bone; Vk, ventral keel.

all specimens partially or completely filling some of the recesses or being sandwiched between the endosteal lamellae of the cavities and the primary bone (Figs 3c,e,f and 4a–d,f). The compaction level of these tissues is variable (see ‘Icsb’ and ‘Ipsb’ in Figs 3 and 4) but always

higher than that of the medullary bone-like tissue with fewer but still wide vascular canals running irregularly (Figs 3e,f, and 4b,c), longitudinally (Fig. 4d) or radially relative to the centre of the cavity (Fig. 4f). These secondary tissues generally show a highly interwoven

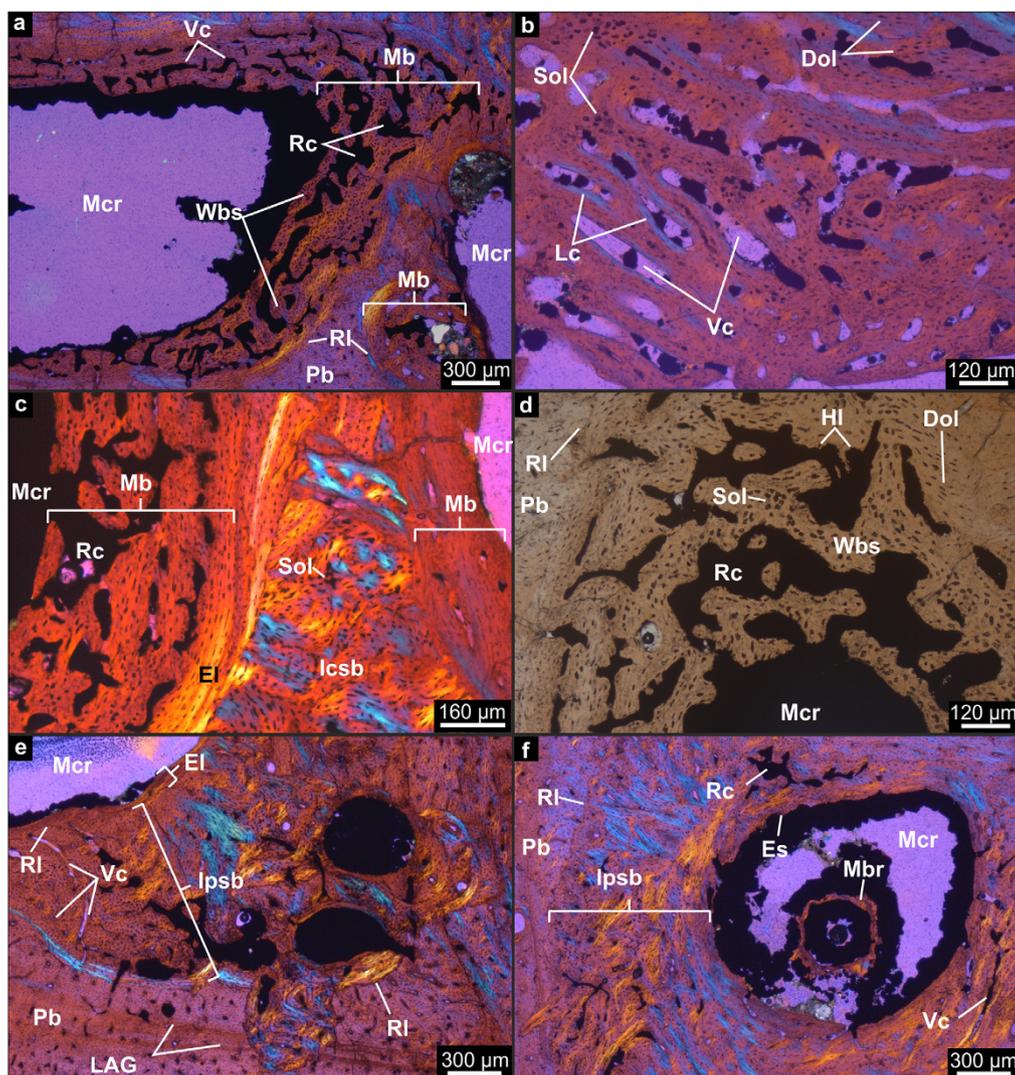


Figure 3 | Microanatomy and histology of the medullary bone-like tissue and the more compacted secondary tissues in multiple sections of 2007.111.1(20). Note that medullary bone-like tissue is in different phases of development: areas of intact bone are seen in (a) and (b); resorption just started in (c) and (d); advanced resorption only leaves a ring of the tissue in the cavity in (f). In (c) the interwoven compact secondary tissue with large SO-lacunae is sandwiched between two recesses showing medullary bone-like tissue deposition, and is separated from them by distinct resorption lines. In (e) and (f), the highly porous, unorganized secondary bone is sandwiched between the endosteal lamellae of a central cavity and the primary cortex. Image in (d) was taken under single plane polarizers; the rest of the images under crossed plane polarizers aided by lambda wave plate. Abbreviations: Dol, dynamic osteocyte lacunae; EI, endosteal lamellae; Es, eroded endosteal surface; HI, Howship lacunae; Icsb, interwoven compact secondary bone; Ipsb, interwoven porous secondary bone; Lc, lamellar compaction; Mbr, remnant of medullary bone-like tissue; rc, resorption channels and cavities; Sol, static osteocyte lacunae; Vc, vascular canal; Wbs, woven bone struts. Other abbreviations as in Fig. 2.

structure containing lacunae of both SO- and DO-derived features (Figs 3c, and 4b,d). They can merge with each other in adjacent cavities and sometimes even with the primary cortex without any sign of a distinct erosion line (Fig. 4d). Thus, their overall appearance is still strikingly different from that of the usual Haversian system, and is more reminiscent of compacted coarse cancellous bone (CCCB). However, the histology and architecture of these secondary tissues show that they mostly grow into the cavities with their own, well organized vascular canal system instead of compacting irregular cavities between already existing trabeculae, as seen in CCCB. It is most likely that these structures in the *Bakonydraco* symphyses are identical to the unusual compacted secondary tissue found sandwiched between layers of lamellar bone in the femur V382 of *Pterodaustro*³⁰.

Discussion

Apart from its non-homologous anatomical location (i.e. not in a long bone), the highly porous, secondary intramedullary bone tissue

found in four symphyses of *Bakonydraco galaczi* (Table 1) looks identical to medullary bone as identified and described in non-avian dinosaurs^{21,27,28}. Based on its microanatomy and microstructure, the formation principles of this tissue is most likely similar to those of fibrolamellar bone (*sensu* Prondvai et al.³⁹) developing in the periosteum of juveniles of fast growing animals^{40–43}. The marked difference is that this tissue grows centripetally within the cavities, mostly on previously resorbed bone surface. Based on its occurrence in multiple individuals in various stages of development, a pathologic origin of this tissue is highly unlikely.

Among more than 30 investigated specimens of the Early Cretaceous pterodactyloid pterosaur, *Pterodaustro guinazui*, Chinsamy et al.³⁰ reported a single femur (V 382), the largest known for the taxon, that showed a considerable amount of endosteally derived bone tissue in the mid-diaphyseal region, which they proposed could represent medullary bone. However, for reasons not mentioned in their work, the authors were not entirely convinced

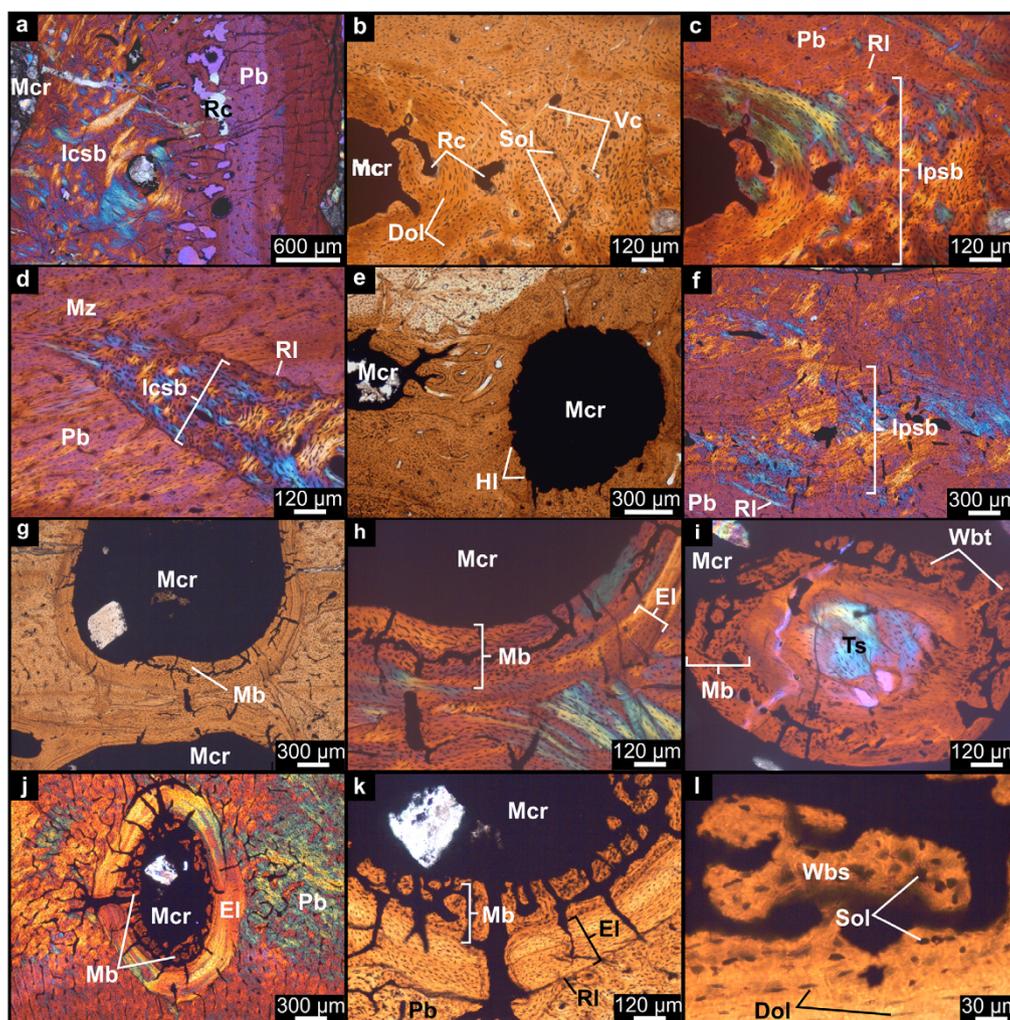


Figure 4 | Medullary bone-like and other special secondary tissues found in other specimens. (a) 2007.111.1(15); (b,c) 2010.74.10; (d–f) 2010.74.3; (g–i) 2010.74.16; and (j–l) 2010.74.18. Beside medullary bone-like tissues, the presence of highly porous and more compacted interwoven secondary bone tissues is very common among all specimens. In (i) medullary bone-like tissue is deposited on a pre-existing trabecular strut that is cut transversely in this section. Images in (b), (e), (g), (k), and (l) were taken under single plane polarizers; the rest under crossed plane polarizers aided by lambda wave plate. **Abbreviations:** Mz, merging zone of the confluent primary and secondary bone; Ts, trabecular strut. Other abbreviations as in Figs 2 and 3.

about the true nature of this tissue³⁰. Unfortunately, we could not properly identify the fine characteristic details on the provided images, either. Even so, related to these findings, identifying medullary bone-like tissues in the mandibular symphyses of *Bakonydraco* raises many important questions.

Could the nature of the described secondary tissues be the same in the two pterosaur taxa and if yes, could they be considered as medullary bone in these pterosaurs? Although found in different anatomical locations, based on the description of Chinsamy et al.³⁰ these

tissues have very similar histological characteristics in *Pterodaustro* and *Bakonydraco*, which speaks for a common tissue type. The justness of referring to them as medullary bone is harder to judge. On a pure microanatomical and microstructural basis, these medullary bone-like tissues appear to meet the criteria characterizing medullary bone. However, when considered functionally, there is substantial evidence against their expected solely reproductive role in pterosaurs for two reasons. First, in *Bakonydraco* primary bone histology indicates that growth rates of 2007.111.1(15), 2007.111.1(20) and

Table 1 | Some details of the sectioned symphyses. Specimens are arranged in ascending ontogenetic rank order from juveniles to adults. Reconstructed symphysis length data and histology-based relative ontogenetic sequence of the specimens are taken from [34]

Specimen ID (MTM V PAL)	Reconstructed symphysis length (mm)	Histologic ontogenetic sequence	Medullary bone-like tissue present(+)/absent(-)
2007.111.1(15)	80.5	1 (juvenile)	+
2010.74.16	124	2 (juvenile)	+
2007.111.1(20)	69	3 (juvenile)	+
2010.74.3	96	4 (juvenile)	-
2010.74.10	111	5 (subadult)	-
2007.111.1(9)	95.5	6 (adult)	-
2010.74.18	126	7 (adult)	+



2010.74.16 were the highest among all symphyses corresponding to the earliest histologic ontogenetic stages in the sequence, with 2007.111.1(15) and 2007.111.1(20) also being the smallest specimens³⁶ (Table 1). Skeletal immaturity alone would not exclude the sexual maturity of these specimens, as for instance, non-avian dinosaurs are believed to have started reproduction well before reaching adult body size^{27,44,45}. However, the observation that the three, apparently fastest growing specimens exhibit medullary bone is exactly the opposite of the expected considerable slowdown of growth due to the redirection of energy from growth to reproduction^{44–47}. Sexual maturity occurring at 53% of presumed adult size was suggested for *Pterodaustro* based on the histological indicators of a significant decrease in bone growth rate⁴⁷. Although it is more likely that this slowdown of growth resulted from the onset of the energy consuming flight rather than that of reproduction⁴⁸, marked decrease in bone growth rate is expected either way. Finally, the only unambiguously sexually mature female pterosaur found so far, a *Darwinopterus* specimen associated with an egg, was considered skeletally mature based on its co-ossified skull and fused postcranial elements⁴⁹.

Second, pterosaurs are believed to have laid eggs with extremely thin, even leathery (“parchment-like”) shell^{49–53}, similar to most oviparous non-gekkonid squamates^{54,55}. Therefore the calcium content of eggshells and hence the calcium demand for their formation in pterosaurs must have been lower than that of the thicker, heavily calcified shells characterizing the eggs of archosaurs and turtles^{56,57} or even the rigid-shelled gecko eggs⁵⁶. Although Chinsamy et al.³⁰ argued that the egg of *Pterodaustro* was shown to have had hard shell⁵⁰, and therefore this genus may have required medullary bone during egg-laying³⁰, it is still valid that the eggshell of *Pterodaustro* was also extremely thin (30 μm)⁵⁰. Thus, pterosaurs apparently would not have needed medullary bone for eggshell production⁵⁸. Nevertheless, developing medullary bone is not a prerequisite for laying thick, hard-shelled eggs, either. Females of examined crocodylian and turtle species do not form medullary bone during their reproductive phase; however, a significant amount of endosteal structural bone is resorbed, whereas periosteal apposition is reduced in egg-laying females^{31,32}. Not even every bird species builds up calcium reservoirs in their bones during the reproductive period. For instance, Pahl et al.²² found neither thickened bone walls, nor medullary bone development in the females of three passerine bird species before and during the laying of a clutch. Thus, the relationship between medullary bone formation and rigid eggshell production seems not as straightforward and unequivocal as it is generally claimed.

Even if not solely for reproduction, medullary bone-like tissues in these pterosaurs certainly had the capacity to serve as a calcium reservoir, but less so to have any biomechanical role. Although Fleming et al.⁵⁹ reported increasing breaking strength due to medullary bone deposition in the humerus of egg laying hens, medullary bone is generally considered a non-structural bone tissue, and thus biomechanical significance of similar tissues in the symphysis related to e.g., feeding habits of *Bakonydraco* is unlikely. As shown by the considerable size differences³⁶ among the specimens displaying medullary bone-like tissues, the formation of this tissue is apparently size-independent further weakening a biomechanical hypothesis. On the other hand, the consistent presence of medullary bone-like tissues in the three, most actively growing specimens may suggest a role in growth dynamics. The high calcium demand during the phase of rapid skeletal growth could have required the development of such fast growing secondary bone as a special reservoir that could have been easily mobilized and thereby ensured the desired calcium supply even in periods of malnutrition or under other unfavourable conditions. This hypothesis on its own, however, is attenuated by the presence of medullary bone-like tissue in the largest, histologically adult *Bakonydraco* mandibular symphysis, and presumably in the adult femur V 382 of *Pterodaustro*³⁰. It seems more likely that

high calcium turnover rate was needed for variable physiological processes in different ontogenetic stages requiring the periodic deposition of medullary bone-like tissues throughout the individual’s life. These processes could have included phases of fast growth, periods of resource limitation, even migration as well as reproduction. The need for depositing a bone tissue of such high recruitment rate at any time in the skull can also relate to the lightweight construction of most postcranial bones in pterosaurs (hollow bones with extreme thin cortices^{58,60}), which therefore may not have been able to sufficiently contribute to the general calcium metabolism without damaging their structural integrity.

The more compacted, interweaving endosteal tissues, which are present in most symphyses, most likely correspond to the unusual compacted secondary tissue described in the femur V 382 of *Pterodaustro*³⁰. Although the microanatomy of these tissues looks less like that of medullary bone, for a secondary tissue unusually high vascularization and all other histological characteristics of fast formation imply that they, too, could have taken part in rapid calcium mobilization. The gradual structural transition of the medullary bone-like tissue into these interweaving endosteal tissues also speaks for a common or at least very similar, most probably physiological role. The similar tissue found in *Pterodaustro* was suggested to represent compacted remains of medullary bone³⁰ as it can be observed in some laying hens⁶¹. In hens, the role of compaction and incorporation of unresorbed remains of medullary bone into the cortex is to compensate for the cortical bone loss in the reproductive phase. This is necessary because apparently not only medullary bone, but also a considerable amount of structural bone is being resorbed during egg-laying in hens⁶¹. If, indeed, the compacted secondary tissues observed in pterosaurs have similar formation principles (as their histological features indicate), resorption of the medullary bone-like tissue may have been accompanied by structural bone resorption in pterosaurs too. Such extensive bone resorption activity that necessitates fast re-deposition of structural bone resulting in these odd secondary tissues also suggests physiological periods of unusually high calcium demand. For whatever reasons, pterosaurs undoubtedly possessed a highly advanced, dynamic bone recycling system that seems to have been active, at least in *Bakonydraco*, throughout their life including early ontogenetic stages.

Another worthwhile hypothesis for the nature of this medullary bone-like tissue is that it represents remnants of alveolar bone. Alveolar bone develops to anchor teeth into the jaw bone, is metabolically very active⁶² consequently having a similar histological appearance to medullary bone (with high porosity, woven bone content, and remodelling rate), and its gross anatomical location matches that of the medullary bone-like tissue in the *Bakonydraco* symphyses. Still, *Bakonydraco* was, as all azhdarchids, edentulous³³, which questions the evolutionary benefits of retaining functionless alveolar bone to such extent as seen in the studied symphyses. It is still possible that alveolar bone had gained some secondary function in this pterosaur. However, in mammals alveolar bone never develops in genuinely toothless areas (e.g. diastema) because the epithelial cells of the developing dental lamina are needed to induce the cranial neural crest cell-derived ectomesenchyme to form the dental follicle which later gives rise to alveolar bone-forming osteoblasts⁶². This further weakens a homologous origin of alveolar bone and the medullary bone-like tissue in *Bakonydraco*. In addition, the presence of medullary bone-like tissues in the sagittally positioned cavities of the mandibular symphyses, including the ventralmost channels, also speaks against an alveolar bone origin. Nevertheless, the capacity of the mandible to form the medullary bone-like tissue in *Bakonydraco* and alveolar bone in other taxa may share some common developmental factors.

The lack of further reports on medullary bone-like tissue in other pterosaurs does not necessarily mean that the occurrence of this tissue is taxonomically restricted. Most studies identifying medullary



bone in dinosaurs (including birds) focused on long bones, even though medullary bone has been reported to be present throughout the skeleton, including the skull, in laying chickens¹⁷. Although no postcranial pterosaur material can be associated with the diagnostic lower jaw of *Bakonydraco galaczi* with certainty⁶³, none of the pterosaur limb bones found in the locality and sectioned so far shows such medullary bone-like tissue as do the symphyses (EP pers. obs.). This is in sharp contrast with the proportionally high frequency of this tissue in the sectioned symphyses (in four out of seven specimens), and suggests a location-specific occurrence of medullary bone-like tissues in *Bakonydraco*. Such a restricted, element-specific distribution can be a reason for the lack of finds among the abundant histological samples of pterosaur long bones^{58,60} where medullary bone-like tissues may simply be less characteristic than medullary bone is for the limb bones of dinosaurs. Possible reasons for such a distribution pattern can include biomechanical or other, yet unknown constraints on the bone wall thickness of the pterosaur postcranial skeletal elements. Therefore, it is possible that histological investigation of some cranial bones of other pterosaurs will reveal such tissues in other taxa, as well.

The ability to periodically deposit endosteally derived secondary bone, the microanatomy and histology of which indicates very high turnover rates, was unquestionably present in pterosaurs and dinosaurs, irrespective of the function and anatomical location of these tissues. Hence, it seems straightforward to conclude that their presence in pterosaurs further strengthens the hypothesis of dinosauromorphs and pterosaurs being closest sister groups^{63–68} (but see Bennett^{69,70} for contrary opinion). However, the apparently very rare occurrence of medullary bone-like tissues in the limb bones of pterosaurs and the presumed differences in their function may suggest independent evolutionary appearances of these tissues in the two clades. Alternatively, the dedicated reproductive function of medullary bone-like tissues could have evolved later on the dinosaurian lineage in which case these tissues may have common, homologous origin with diverging functions and physiological regulatory systems. A common evolutionary origin may gain some support by the findings of Cerda and Pol⁷¹ who revealed medullary-bone like tissue in the basal sauropodomorph *Mussaurus patagonicus*, albeit in a recent study Cerda et al.⁷² reconsidered it as a pathologic condition possibly originating from avian osteopetrosis. Although close-up images of the lacunar features were not provided, the described fine-scale histological characteristics based on which they argued for this tissue to be distinct from medullary bone were its high density of large osteocyte lacunae and the lamellar coating of its vascular canals⁷². These features, however, match the characteristics of the apparently non-pathologic medullary bone-like tissues in the *Bakonydraco* symphyses. In accordance with the original interpretation⁷⁰, this resemblance raises the possibility that the strange tissue in *Mussaurus* may not be pathologic but instead shows an evolutionary earlier stage of histological appearance with unknown functional aspects.

The gradual evolutionary change hypothesis leads back to the problem of calling such tissues medullary bone in extinct animals. On one hand, if we accept that medullary bone can be identified by its microanatomical and histological features without having firm evidence of its reproductive function, as is the case in effect in all extinct taxa, then pterosaurs apparently possess medullary bone which may not be used as an ultimate indicator of sexual maturity in these animals. On the other hand, if the term medullary bone is also restricted by its reproductive function along with its hormonal regulatory system as described in birds, then similar tissues cannot be unambiguously identified as medullary bone in any extinct taxon, because we have no direct evidence of these characteristics. Although the dinosaurian nature of birds still strongly supports the reproductive role of medullary bone in non-avian dinosaurs, the general concept that medullary bone-like structures develop only in female

animals being in their reproductive (egg laying) cycle, as in birds^{21,27–29} should be considered more circumspectly.

In any case, our findings strongly encourage further research on the potential alternative functions as well as evolutionary origin, distribution, and significance of medullary bone-like tissues.

Methods

Along with more than fifty other specimens³⁶, the seven mandibular symphyses of the azhdarchid pterosaur *Bakonydraco galaczi* studied here were found at the Iharút vertebrate locality, in the Upper Cretaceous (Santonian) layers of the Csehbánya Formation³³. All specimens belong to the Vertebrate Paleontological Collection (V PAL) of the Hungarian Natural History Museum (MTM) and are referred to only by their specimen numbers in the text. Preparation was carried out as described by Prondvai et al.³⁶

The seven mandibular symphyses studied here were all sectioned and histologically investigated by Prondvai et al.³⁶ who reconstructed the relative ontogenetic sequence of the specimens with qualitative and quantitative histological methods. The reconstructed length and ontogenetic sequence of the specimens supported by most of the histological analyses in the latter study is shown in Table 1.

After recognizing medullary bone-like tissues in one of the cross sections prepared for a previous study³⁶, additional cross and longitudinal thin sections were prepared of all specimens for the current study using the same methods (Fig. 1). Histological features of the thin sections were examined under Nikon LV 100 polarized light microscope (Nikon Corp., Tokyo, Japan). Pictures of the thin sections were taken with a QImaging MP5.0 digital microscope camera (QImaging Corp., Surrey BC., Canada) and processed with Image Pro Insight 8.0 (Media Cybernetics L.P., Maryland, USA) software.

Histological descriptions and tissue identification follow the concept of Stein and Prondvai³⁸ and Prondvai et al.³⁹, vascular architecture categories that of Francillon-Vieillot et al.⁷³. The current study focuses on the secondary bone structures found in these specimens; primary tissues have already been described in detail by Prondvai et al.³⁶. Medullary bone-like tissues are evaluated following the criteria applied for medullary bone in other fossil taxa^{21,27,28}.

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Author contributions

E.P. and K.S. designed the research, performed the investigation and discussed the results; E.P. performed the artwork and wrote the paper.

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