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.

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 ostrich10,11,17–21. 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 cycle10,11,17,21,22. Apart from the controversial results on mice forming medullary bone-like tissues in response to unnaturally high oestrogen doses20,24, medullary bone has not been reported in any extant non-avian amniotes20,26. 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 dinosaurs24,27–29. 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 bone29. Since it has been found in theropod24,27–29 as well as in ornithopod24,28 dinosaurs but not in extant alligators21,32, medullary bone has been suggested to have first appeared in the ornithodiran lineage of archosaurs after its divergence from crocodilians27,32.
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 Hungary. 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 lizards and crocodiles. This refers to a sutural region, as seen in the variety of syndesmoses characterizing fibrous connection or interdigitating arrangement of bone in the physes are completely ossified without any histological sign of former.

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The secondary tissues, less reminiscent of medullary bone in their overall morphology but still considerably perforated, can be found in...
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...
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...
about the true nature of this tissue\(^{29}\). 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.\(^{29}\) 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 specimens** (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**. 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**. 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 rate**. Although it is more likely that this slowdown of growth resulted from the onset of the energy consuming flight rather than that of reproduction**, marked decrease in bone growth rate is expected either way. Finally, the only unambiguously sexually mature pterosaur found so far, a *Darwinopterus* specimen associated with an egg, was considered skeletally mature based on its co-ossified skull and fused postcranial elements**.

Second, pterosaurs are believed to have laid eggs with extremely thin, even leathery (“parchment-like”) shell**-**, similar to most oviparous non-gekkonid squamates**-**. 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**-** even or even the rigid-shelled eggs *Gekko***. Although Chinsamy et al.** argued that the egg of *Pterodaustro* was shown to have had hard shell**, and therefore this genus may have required medullary bone during egg-laying**, it is still valid that the eggshell of *Pterodaustro* was also extremely thin (30 μm)**. Thus, pterosaurs apparently would not have needed medullary bone for eggshell production**. Nevertheless, developing medullary bone is not a prerequisite for laying thick, hard-shelled eggs, either. Females of examined crocodilian 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**. Not even every bird species builds up calcium reservoirs in their bones during the reproductive period. For instance, Pahl et al.** 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.** 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 symphyses related to e.g., feeding habits of *Bakonydraco* is unlikely. As shown by the considerable size differences** 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***. 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**), 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***. 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 bone** as it can be observed in some laying hens**. 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 hens**. 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 active** 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, edentulous**, 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 osteoblasts**. 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
This resemblance raises the possibility that the strange tissue in symphyses. In accordance with the original interpretation, bone-like tissues in the Bakonydracon. Such a restricted, element-specific distribution can be a reason for the lack of finds among the abundant histological samples of pterosaur long bones 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 (but see Bennett 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 dinosauromorph 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 Pol who revealed medullary-bone-like tissue in the basal sauropodomorph Musaurus patagonicus, albeit in a recent study Cerda et al. 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 canals. These features, however, match the characteristics of the apparently non-pathologic medullary bone-like tissues in the Bakonydracon. In accordance with the original interpretation, this resemblance raises the possibility that the strange tissue in Musaurus 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 dinosauromorph 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 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 specimens, the seven mandibular symphyses of the azhdarchid pterosaur Bakonydracon were studied here. These are published by a paper in 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.

The seven mandibular symphyses studied here were all sectioned and histologically investigated by Prondvai et al. 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 pathological 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 study, 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 (Q Imaging Corp., Surrey BC, Canada) and processed with Image Pro Insight 8.0 (Media Cybernetics L.P., Mountain, USA) software.

Histological descriptions and tissue identification follow the concept of Stein and Prondvai and Prondvai et al., vascular architecture categories that of Francillon-Viellet et al. 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. Medullary bone-like tissues are evaluated following the criteria applied for medullary bone in other fossil taxa.

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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.

Additional information

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