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**CONTENT**



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We thank all the reviewers for their effort and time invested to improve the papers published in this issue.

# Rare dental anomalies in two sympatric European bat species (*Pipistrellus* spp.)*

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## Abstract

Over the past twenty years, we documented striking non-lethal anomalies in the dentition of the common pipistrelle (*Pipistrellus pipistrellus*) during field work in various parts of Germany. In 2019 we got knowledge of two bat nurslings (*P. pipistrellus*, *P. pygmaeus*) from the Rhine-Main area (Hesse, Germany) with ultimately fatal dental anomalies. The aberrations were documented with high-resolution X-ray techniques. Here we discuss our results in the context of published, but mostly minor and obvious non-lethal teeth anomalies in bats.

**Keywords:** dental anomalies; high-resolution 2D-X-ray; micro-computed tomography; *Pipistrellus* spp.; Chiroptera

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## Introduction

Dental anomalies in bats (Order Chiroptera) have been mentioned in the review literature during the last decades (1-5). Dental variation or an abnormal number of teeth can result in an increased number (polyodontia, hyperdontia, supernumerary or extra teeth) as well as the opposite (oligodontia, hypodontia, missing teeth). Most of this information derives from museum collections, which provide an invaluable source of data (1-3, 6-8). Almost all this data report on dental anomalies of the small teeth (incisors, premolars).

Even during fieldwork single bat individuals with minor teeth alterations have been detected and collected: an adult male of *Carollia brevicauda*

was caught in Columbia with an additional left and right incisor in the maxilla which had separate alveoli (bilateral hyperdontia (5), and an adult male individual of *Sturnira lilium* mist-netted in southern Brazil showed a diastema between the upper incisors (9). Among 33 individuals from *Artibeus lituratus* polyodontia and oligodontia in the incisors occurred in one case each. This is remarkable because it was a single population (10). In the Palearctic a decrease in the number of small premolars has been reported in different *Myotis* species, while additional premolars seem to appear rarer (11).

Periodontal disease which may result in teeth loss is also known in bats (12). In museum collections of the insectivorous bat *Myotis daubentonii* severe periodontosis was reported, one that led to the loss of all upper and two lower molars (8, p. 107). Severe periodontosis is also mentioned to be quite common in *Pipistrellus pipistrellus*, which even could be up to 50% among perennial adults in (2, p. 787). An extreme periodontal disease was reported from *Tadarida brasiliensis* in Texas (13), which resulted in loosing most of the bat's teeth.

Here we also introduce other minor teeth alterations of unknown reasons found in living bats in the field, which were released after documentation (Figure 1). In contrast to these minor teeth alterations, we report on two lethal cases in *Pipistrellus* species: a common pipistrelle (*P. pipistrellus*, Figure 2-4), which had no visible teeth at all and a soprano pipistrelle (*P. pygmaeus*, Figure 5-8), which showed an aberration in the right upper and lower jaw including missing teeth. Both species are widely distributed in Germany (and in Europe). They are quite common and usually occupy urban areas. Since the 1990es, differences in echolocation as well as the social calls of what were once considered a single species were detected (14-18), with the description as separate species in 2001 (19).

## Material and methods

Anomalies in the dentition of the common pipistrelle (*Pipistrellus pipistrellus*) were documented during field work (including capture of living bats by mist netting) between 1986 to 2019. From this archive of field notes and photos we extracted the field information while we got the two nurslings via contacts to the public and from very experienced volunteers in raising injured or weakened bats. All measures taken in the field complied with the respective species and animal protection laws; appropriate permits were obtained.

We carried out in-depth examinations in two specimens: (1) *Pipistrellus pipistrellus* (SMF 98603, Senckenberg Frankfurt mammal collection), subadult female found weakened in July 2019 in Frankfurt/Main, and (2) *Pipistrellus pygmaeus* (SMF 98604, Senckenberg Frankfurt mammal collection), subadult male, weight 2.7 g, found weakened in August 2019 in Münster, 30 kilometres south of Frankfurt/Main. We examined the defects in the skulls of both animals non-destructively using high-resolution 2D and 3D X-ray methods. 2D-radiographs of the bats were done at the Senckenberg 2D-Xray Lab in Frankfurt/Main (SGN-SF-2D-Xray-Lab) on digital imaging plates with the Faxitron HP (50 kV, 3 mA, exposure time 60 or 90 s). They were read in with a DÜRR laser scanner with a resolution of 7 µm (which is 3,809 dpi). The subsequent image processing was done with Photoshop CS6, whereby the 16 bits images allow first a linear, then a sigmoid gradation adjustment.

For micro-computed tomography, both bats were scanned at the Senckenberg CT-Lab Frankfurt/Main with the ProCon-X-Ray-Micro-CT with a 100 kV Finefocus tube (SGN-SF-3D-Xray-CT). The scans were performed with 90 kV and 89 µA, generating 2,400 projections with 1,500 ms per scan. To increase the signal to noise ratio, three exposures per image were averaged. While recording, the ring artefact reduction mode was applied. The resolution (voxel size) was 7.5 µm. Both data sets were segmented and visualized with VGStudio MAX, version 3.4.5 (Volume Graphics, Heidelberg, Germany).

Abbreviations: Tooth positions are indicated by letter and number, upper case indicating upper tooth (e.g., M1 is upper first molar), lower case indicating lower tooth (e.g., m3 is lower third molar).

## Results

During field research we caught bats with mist nets and repeatedly found animals with slight non-lethal dentition changes, as can be seen for example in Figure 1. These animals showed massive deposits (tartar or dentine) each on the upper left canine, the premolars and – particularly clearly – the first molar (M1). Additional hard material of unknown composition was found at the gums, one that could not be removed with tweezers. Showing no sign of weakness or disease, they were released after being netted.

We examined and X-rayed about one hundred bodies from bats of different species from Germany, mostly Hesse. Most of them originated from bat rehabilitation. Only two of them had non-accidental but lethal deformations.

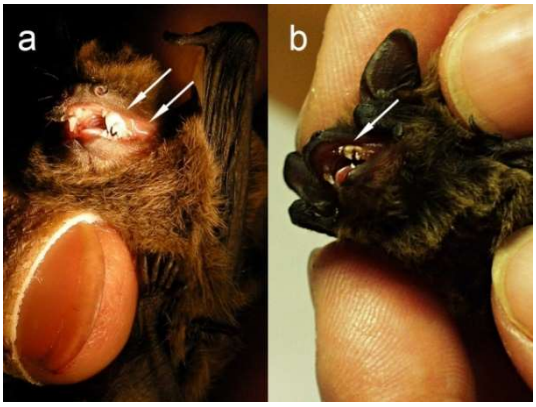


**Figure 1. Examples of teeth anomalies (arrows) of *Pipistrellus pipistrellus* documented during fieldwork in (a) Saxony-Anhalt 2003 and (b) Lower Saxony summer 1999. Both bats had massive deposits on the upper left teeth, especially on the first molar. A few bats also showed some hard material at the gums (a).**

The first one is a female common pipistrelle (*Pipistrellus pipistrellus*) without visible teeth, found weakened on the ground by private people on July 11, 2019 in Frankfurt/Main, died July 16, 2019. When receiving the animal, the throat and tongue were already discoloured black due to lack of blood oxygen, the tooth tips of the molars that have not erupted can only be guessed at through light reflections in the inset of Figure 2. The bat rejected insects as food, but happily ate liquefied mealworms or puppy milk. Our examinations showed that all teeth are present (Figs. 2 and 3). The complete permanent dentition is developed, and all molars are calcified (Figure 3). The skull length of this bat is about 1 cm with the upper right molars marked with M1 to M3. For better visibility, an extra colour (duplex, blue) was digitally added to the radiograph. The age of the common pipistrelle is subadult as all permanent teeth are erupted, including M3s which are the latest to erupt, and the epiphyses of the humeri are not completely fused and show cartilage.

The second bat is a male soprano pipistrelle (*Pipistrellus pygmaeus*) found lying on the ground on August 16, 2019, at Münster, around 30 km south of Frankfurt/Main, Hesse, which survived under human care until October 1, 2020. Figure 4 shows a severe malformation of the right upper jaw and an undeveloped right eye, while Figure 5 gives a look into the open mouth of the living animal and shows the massive palatal deformation on the right side. These anomalies of the bony structures are clearly shown in 3D reconstruction from the tomography cross-section images (Figure 6). Moreover, there is an isolated solid substance, presumably dentine, because of same grey value, visible on the right side next to the palatal deformity.

This soprano pipistrelle reported here in detail lacks teeth in various positions. The dental formula in the genus *Pipistrellus* reads (2, p. 757)

| | | | |
|---|---|---|---|
| I 2 | C 1 | P 1-2 | M 3 |
| i 3 | c 1 | p 2 | m 3 |

Thus, the complete number of teeth is 34, whereby according to the literature (2, p. 757) the upper first, very small premolar can sometimes be missing. The examined bat has 27 teeth only (Figure 7). As expected, the complete dentition is found in the left upper jaw (n=8, Figure 7d), which shows no external impairment, but surprisingly also in the right lower jaw (n=9, Figure 7f). The cingulum of the lower second molar (m2) has developed a protuberance.

As expected, most of the teeth are missing in the upper right jaw, namely all incisors, the canine, and the second as well as the third molar (M2 and M3). With the first molar (M1) and the two premolars, there are only three instead of eight teeth (Figure 7a-c). Surprisingly, two incisors are also missing in the lower left jaw (Figure 7e), so that there are only seven teeth in total. It is remarkable that – contrary to the literature for *Pipistrellus* – all premolars are present in this bat. The small first upper premolar (P3) has one root, the larger second premolar (P4) has three roots (Figure 7a-b).

We cannot say for sure how old the animal was when it was found. The tomography was only performed after the natural death of the meanwhile adult male, that survived 13 months in human care. However, the circumstances of the find suggest that it was also a subadult animal at that time. It probably landed exhausted on the ground due to lack of food during the first independent hunting flights and was found there (pers. comm. D. and S. Diehl).

In tomography, the total height of the skull of the soprano pipistrelle comprises 1,024 projections.

With a voxel size of 7.5 µm, this corresponds to a total skull height of 7.7 mm.

**Figure 2. Radiography of common pipistrelle (P. *pipistrellus*, SMF 98603) X-ray shows complete dentition (inset: head).**

The sectional images show the skull structure like that in histological sections, but non-destructively. From 1,024 layers in total, three are shown in Figure 8. They illustrate the extent of the deformation in different virtual layers of the skull: The intact left side of the skull (Figure 8a) shows the frontal teeth (I1+I2, C) of the upper jaw as well as the roots of P3 to M3. Figure 8b shows the situation 225 µm higher: The frontal part of the right maxilla is missing, the rest of the bone is blistered and swollen because of a long-lasting inflammatory process with bone remodelling. The maxillary bone is significantly thickened, mainly on the right, but also on the left side of the maxilla. Signs of infection are visible throughout the facial skeleton (Figure 8c; 450 µm higher). On the right side (= left in the picture) the isolated dentine is visible. The left eyeball is clearly visible in tomography, on the right it is not developed (see Figure 4).

## Discussion

As far as we know there are no non-fatal dental anomalies reported such as those documented by us during the field work. They may have been observed but not documented or not considered worthy of publication.

In our literature research, we did not find a single reference for the common pipistrelle (*Pipistrellus pipistrellus*) reporting all teeth present but unerupted. This may be since the animals are very small and bat workers do not always have access to laborious methods, such as high-resolution X-ray, to detect these anomalies.

On the other hand, there are several publications on missing teeth. As shown in the introduction, most of the anomalies described relate to the loss of single, mostly small, teeth due to natural causes or accidents. In contrast, reports of the loss of molars (1) or numerous teeth are very rare (13).

Phillips & Knox Jones Jr. (1) reported among 150 examined individuals of the north American insectivorous bat *Pteronotus psilotis* only one adult male lacking an upper molar as well as a first and third lower premolar from disease or mechanical damage. Therefore, we can state unequivocally that the two lethal anomalies we have described here – of which *P. pygmaeus* with the missing teeth resembles the one described above – have nothing to do with accidents.

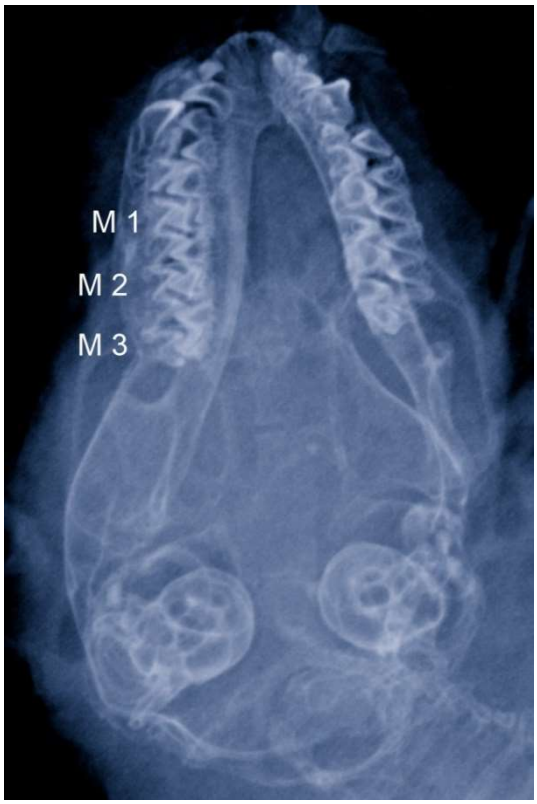


**Figure 3. Radiography of the skull of P. *pipistrellus* (from Figure 2), length about 1 cm.**

Figure 4. Soprano pipistrelle (*P. pygmaeus*, SMF 98604) with a severe malformation of the right side of the head including missing eye. Photo: Birgit Emig.

Figure 5. *P. pygmaeus* (same individual as shown in Fig. 4) with partially missing upper jaw. Photo: Hans Schwarting

A second and most severe case of tooth loss is reported from a male of the insectivorous bat *Tadarida brasiliensis* in Texas, having severe inflammation of its gums and had only eight teeth left (13): I2 and C in the right upper jaw, P4 in the left upper jaw, i1, i2, c, m2 and m3 in the left lower jaw and nothing left in the right lower jaw. Nevertheless, these remaining eight teeth – instead of normally 30-32 – did enable successful

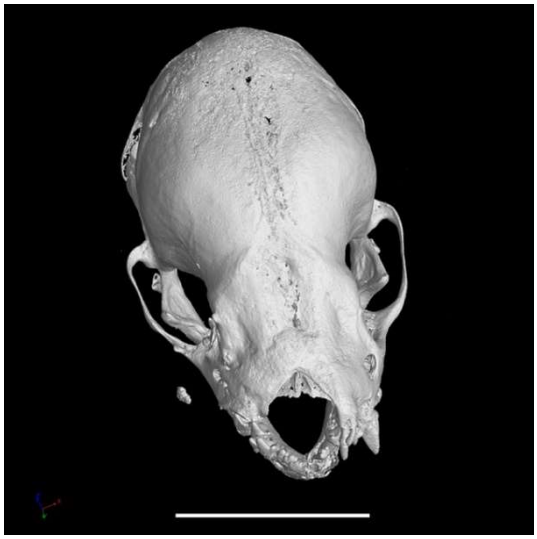

Figure 6. Skull of *P. pygmaeus* (from Fig. 4 and 5) as 3D reconstruction of computer tomography images showing in detail the deformation of the maxilla. Also note the isolated dentine. Scale 5 mm.

hunting and feeding, as the body weight at the time of capture was within the average variation. In our case, *P. pygmaeus* had no obviously inflamed gums when it was found (see Figure 5), but was clearly underweight at 2.7 g. The bat recovered excellently in human care through the provision of sufficient and balanced food and was extremely vital until shortly before its death. It appears to have been adequately nourished during lactation and underwent normal development. It is not possible to say when the inflammation developed in the gums and spread to the bone, which was marked by severe inflammatory processes at the time of death (Figure 8). In order not to stress the exhausted animal even more, no examination was carried out after the find. It cannot be ruled out that the basis of the inflammation in the mouth and nose was laid by milk residues while being nursed.

We are aware that small samples like ours can lead to misinterpretations of anomalies in bats (20). Several hypotheses for dental variation are published (most recent compilation see [5]), i.e., specific, or isolated mutations in genes, the appearance and subsequent development of an additional tooth germ, caused by physical damage or trauma, embryological abnormalities, limited gene flow or environmental instability. To name the causes of the anomalies described here would be purely speculative. It is noticeable however that urban species are probably more affected by dental anomalies than forest bats. On the other hand, urban bats are more likely to end

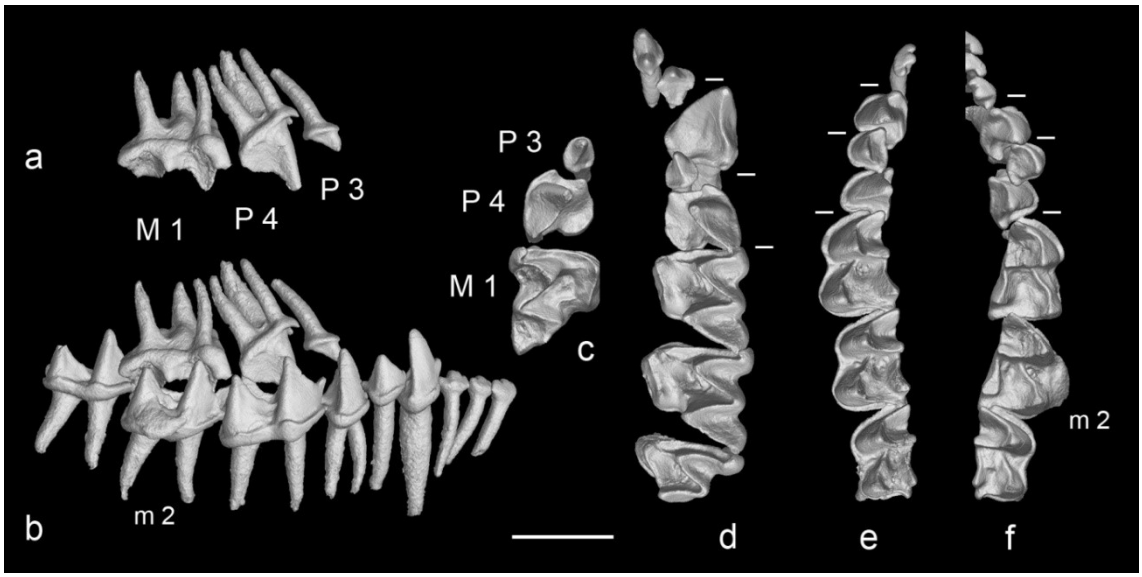


**Figure 7. Complete dentition of *P. pygmaeus* (from Figs. 4-6) with remarkable teeth (see text) labelled with their positions: (a) right upper teeth, (b) right upper and lower teeth in occlusion; both in buccal view, (c) right and (d) left upper teeth, both in occlusal view, (e) left and (f) right lower teeth, also occlusal view: Note the enlarged cingulum of m2. Incisors, canines, premolars and molars separated by lines. Scale 1 mm.**

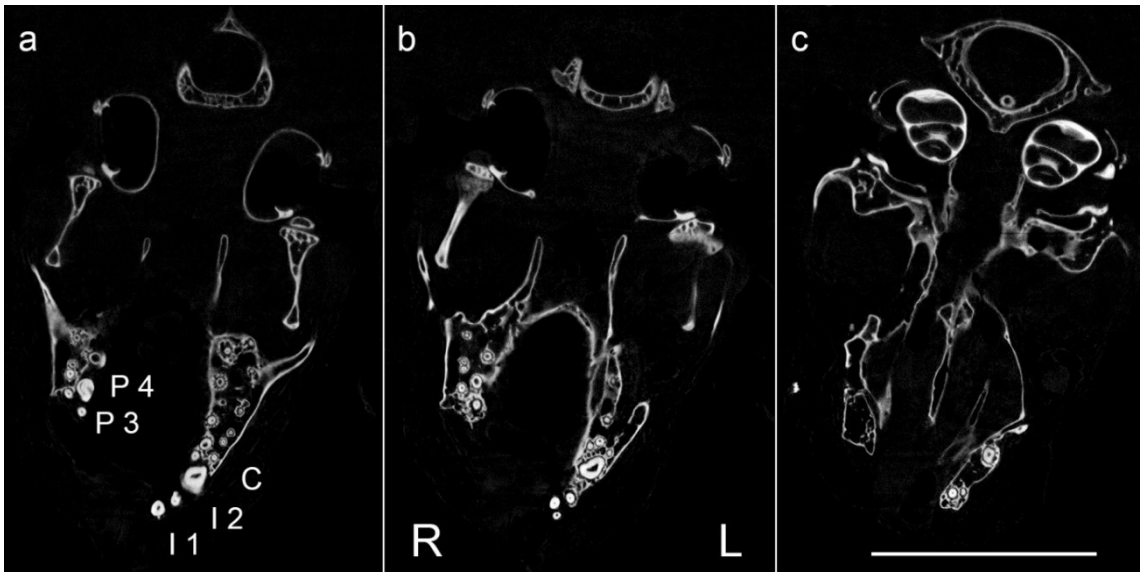


**Figure 8. Sectional images of the skull of *P. pygmaeus* in different planes from below (basal) to above (parietal) show clearer a blistered distended bone than Fig. 6. In (a) both premolars of the right upper jaw are marked. In the left jaw both upper incisors and the prominent canine are labelled, which are missing in the right maxilla. (b) The right maxilla is thickened, in a lesser extent also the left maxilla. (c) Due to the long-lasting inflammatory process the facial skeleton is blistered. Scale 5 mm.**

up in foster care than forest bats and most of our animals came from foster homes. We cannot give a final statement on the reason of the dental anomalies we described here, until more individuals are available and investigated in detail, but we do not exclude current environmental conditions. After the bat population in Germany had demonstrably

stabilized since the 1980s, habitat changes (intensified agriculture, wind turbines, modern profit-oriented silviculture with the removal of damaged trees, urbanization, energetic refurbishment of buildings) and especially, insect mortality pose new threats for these insectivorous animals (21).

Unfortunately, there is a lack of systematic investigations as to whether these tooth anomalies and diseases are normal evolutionary processes or related to the current environmental conditions. Therefore, all X-rayed bats of our study will be kept deep-frozen in the Senckenberg collection (Department Messel Research and Mammalogy) until the end of the project for further investigations (e.g., DNA, toxicology, European Bat Lyssa Virus = bat rabies). We presented this case study here since literature is now more easily accessible worldwide through repositories and publications that refer to local populations are also included in comprehensive literature searches with appropriate keywords and used further, e.g., (5). Additionally, we want also to raise awareness in these anomalies to achieve more information for the scientific community. We plan a study of the large bat collection at the Senckenberg Research Institute, but we will also rely on further material collected from various volunteers in bat protection, from veterinarians and field scientists, who hopefully find handicapped individuals before their natural predators i.e., small carnivores (2, p. 891, 2, p. 652; 22, p. 43) will find and feed on them.

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**Declarations of interest**

None.

**Author contributions**

D.S. collected the data during long-term fieldwork and collected additional material (= dead bats) over many years, R.R. did the radiological investigation (2D, 3D). R.R. and D.S. analysed the material, discussed the results, and wrote the manuscript.

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