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DISEASE IN WILDLIFE OR EXOTIC SPECIES

Dental and Temporomandibular Joint Pathology of the Grey Wolf (*Canis lupus*)

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Summary

Skulls from 392 grey wolves (*Canis lupus*) were examined macroscopically according to predefined criteria. Two hundred and seven skulls were included in this study, comprised of 124 young adults (59.9%) and 83 adults (40.1%); of these, 65 (31.4%) specimens were from male wolves and 104 (50.3%) were from females, with 38 (18.4%) of unknown sex. Out of 8,694 possible teeth, 8,339 (95.9%) were present for evaluation. Fifty-five teeth (15.5%) were absent congenitally, 30 (8.5%) were lost during life and 270 (76.1%) were lost artefactually *post mortem*. Skeletal or dental malocclusion was present in 37 specimens (17.9%), with level bite being the most commonly encountered malocclusion. Enamel hypoplasia was present in five skulls (2.4%), affecting eight teeth (0.1%) in total. An abnormal number of roots was found on 23 teeth (0.3%) on 13 skulls (6.3%). Persistent deciduous teeth occurred in two (1.0%) specimens, affecting one (0.01%) tooth each. Fenestration or dehiscence was found associated with 203 teeth (2.4%) in 72 skulls (34.8%). Periodontitis was noted on 115 skulls (55.6%) and 1,000 teeth (11.5%), affecting significantly more adults ($n = 63$, 75.0%) than young adults ($n = 52$, 41.9%; $P < 0.0001$). One hundred and sixty-one skulls (77.8%) showed signs of endodontal disease, including attrition or abrasion on 144 skulls (69.6%) and 2,522 teeth (30.2%) and 424 fractured teeth (5.1%) on 103 skulls (49.8%). Both lesions affected significantly more adults than young adults. Overt periapical disease was associated with six teeth (0.1%) distributed across five skulls (2.4%). A carious lesion was present on one tooth (0.01%) of one specimen (0.5%). Lesions consistent with temporomandibular joint (TMJ) osteoarthritis were found in 24 specimens (11.6%), affecting 38 joints (9.2%). Trauma to the skull, such as bite marks, bullet holes or blunt trauma, was noted in 44 skulls (21.2%). The grey wolf and the domestic dog *(Canis lupus familiaris)* share common dental diseases; however, the proportion and severity may vary. Although the clinical significance of dental and TMJ pathology in the grey wolf remains unknown, based on the impact of these disorders on the domestic dog, the occurrence and severity of these lesions are likely to play an important role in the morbidity and mortality of this wild canid species.

Keywords: Canis lupus; dental pathology; grey wolf; temporomandibular joint pathology

Introduction

The grey wolf (*Canis lupus*) was first recognized by Linnaeus in 1758 (Linnaeus, 1758). It is the largest member of the family Canidae, with adult males weighing 20–80 kg and measuring 1.27–1.64 m in total length and females weighing 18–55 kg, measuring 1.37–1.52 m (Mech, 1974). It is one of the few top predators surviving the Late Pleistocene megafaunal extinctions (Leonard *et al.*, 2007). As of 2005, 27 New World and 10 Old World subspecies of *C. lupus* are recognized (Wozencraft, 2005), but debates regarding classification are ongoing. As a species, *C. lupus* includes the dingo (*C. lupus dingo*) and the domestic dog (*C. lupus familiaris*). Their original worldwide

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range has been reduced by approximately one-third due to human influence. While North American wolves have a fairly even distribution throughout Canada and Alaska, European and Asian wolf populations are often genetically isolated and thus show reduced genetic variability (Randi *et al.*, 1995; Ellegren *et al.*, 1996; Vilà *et al.*, 1999). Although at the regional level some populations are regarded as severely threatened, the overall population is stable and is classified as least concern by the International Union for Conservation of Nature (The IUCN Red List of Threatened Species, 2017).

Four subspecies occur in Alaska: the Alexander Archipelago wolf (*C. lupus ligoni*), the Northwestern wolf (*C. lupus occidentalis*), the Yukon wolf (*C. lupus pambasileus*) and the Alaskan tundra wolf (*C. lupus tundrarum*) (Wozencraft, 2005). The Alaskan grey wolf population is estimated at 7,000–11,000 individuals. They are found throughout the mainland, most major islands of southeast Alaska and Umiak Island and inhabit about 85% of Alaska's territory (Alaska Department of Fish and Game, 2017).

Wolves are pack animals of varying numbers, usually consisting of 2–12 members, including an alpha male and female. A wolf pack can inhabit a territory of up to 7,770 km^2. They may travel more than 32.2 km per day in search of prey (Fuller, 1989; Mech *et al.*, 1998; Mech and Peterson, 2003). The size of the territory and the daily movements of the pack are dependent on the prey abundance, habitat type and time of year. Their diet is extremely variable, depending on their habitat. Wolves are considered intelligent and most often hunt as a team, which allows them to take prey much larger than themselves. As one of the world's top predators, the majority of their diet comprises large mammals, such as moose (*Alces alces*), bison (*Bison bison*), elk (*Cervus canadensis*), caribou (*Rangifer tarandus*), Dall sheep (*Ovis dalli*), mountain goat (*Oreamnos americanus*), mule deer (*Odocoileus hemionus*), harbour seal (*Phoca vitulina*) and beaver (*Castor canadensis*), but also smaller mammals, such as voles and lemmings (*Arvicolinae* spp.), squirrels (*Sciuridae* spp.), hares (*Lepus othus*, *Lepus americanus*), birds and fish, as well as livestock, carrion and garbage (Peterson and Ciucci, 2003; Wright, 2011; Mech *et al.*, 2015). Some packs leave their regular territory to follow migrating herds or to move into areas with abundant prey such as salmon (*Oncorhynchus* spp.) streams during the spawning season (Mech *et al.*, 1998; Szepanski *et al.*, 1999; Darimont and Reimchen, 2002; Darimont *et al.*, 2003, 2008; Adams *et al.*, 2010).

The dental formula of wolves is the same as for all canids: I 3/3, C 1/1, P 4/4, M 2/3 (Fig. 1). The permanent dentition replaces deciduous teeth at 16–26 weeks of age. Wolf skulls can be distinguished from dog skulls by their orbital angle of 40–45°, as compared with dogs having 53–60° (Mech, 1974). Wolves and other canids that hunt in groups for larger prey than themselves, such as the African wild dog (*Lycaon pictus*) and dhole (*Cuon alpinus*), have relatively large canine and incisor teeth, a longer cutting blade on their mandibular first molar teeth and reduced grinding areas on post-carnassial teeth than canids preying on smaller animals, such as coyotes or foxes. As high rostral bite forces are necessary in the killing and dismembering of large prey animals, it has been suggested that both canine and incisor teeth are extremely important and are required to withstand high bending forces (Van Valkenburgh, 1996). A bladed talanoid of the mandibular first molar tooth (known as the 'trenchant heel') as seen in other large-prey canids is only partially developed in the grey wolf (Van Valkenburgh, 1991). The presumed advantage of an increased cutting blade length is improved feeding performance. The greater reduction in post-carnassial grinding area, together with other anatomical features, such as a broader snout and a wider occiput, might similarly be the result of selection for increased bite forces. By decreasing the length of the post-carnassial dentition, the canine teeth are brought closer to the temporomandibular joint (TMJ), thereby increasing the mechanical capacity of the temporalis and masseter muscles (Van Valkenburgh and Koepfli, 1993). The jaw of a grey wolf with a body weight of approximately 35 kg can exert 493.5 Newtons (N) at the tip of the maxillary canine tooth and approximately 773.9 N at the centre of the maxillary fourth premolar tooth, compared with a dog of 25 kg that exerts 351.5 N and 549.8 N, respectively (Christiansen and Wroe, 2007).

The grey wolf is considered the closest relative of the domestic dog and its dental pathology has been described previously in variable depth for specific groups of this species. These pathological changes include, but are not limited to, acquired tooth loss, fractured teeth, abrasion, caries, enamel hypoplasia, maxillofacial trauma, skeletal/dental malocclusion and supernumerary roots, as well as oligodontia (Van Valkenburgh, 1988; Hell, 1990; Vilà *et al.*, 1993; Pavlović *et al.*, 2007; Räikkönen *et al.*, 2013; Losey *et al.*, 2014). This study was conducted in order to evaluate the nature and prevalence of dental and TMJ pathology of an Alaskan

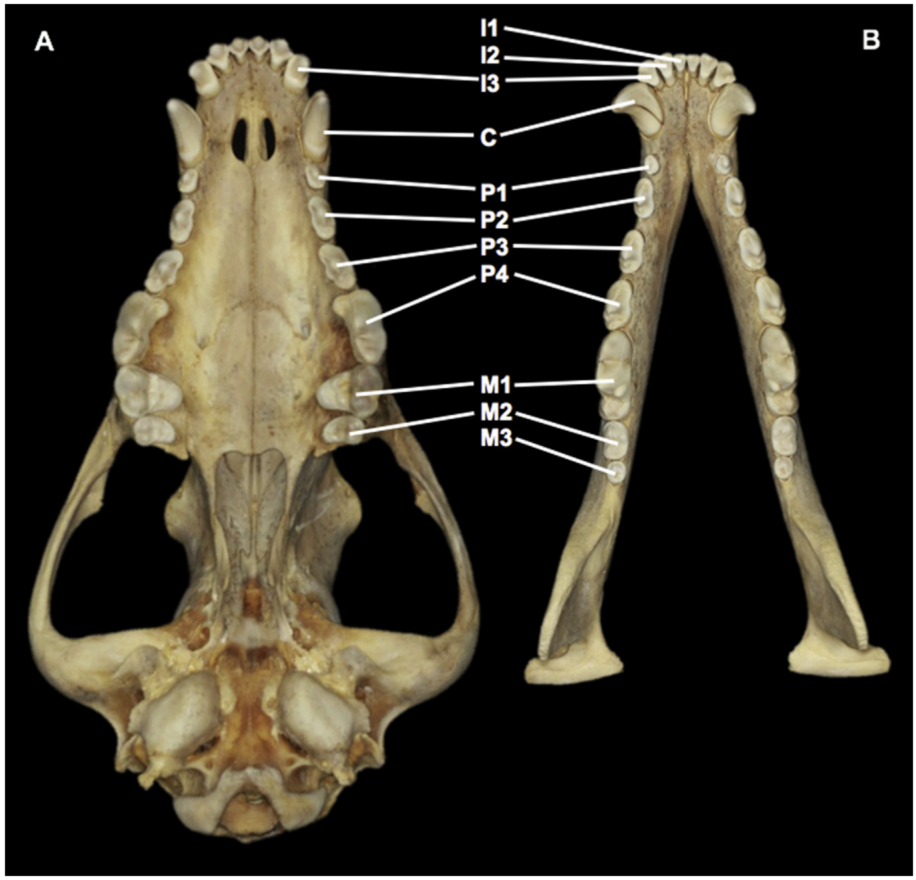

Fig. 1. Representative dentition and occlusion of the grey wolf (UAM 101238) on the maxillae (A) and mandibles (B).

population of grey wolves in museum specimens based on predefined criteria.

Materials and Methods

Macroscopical examination of 392 skulls from the Department of Mammalogy, Museum of the North, University of Alaska, Fairbanks, was performed. Each specimen was labelled with a unique catalogue number and the specimen's sex (if known), collection location and collection date. 'Adult' versus 'young adult' categorization was determined by prominence of cranial sutures. 'Juvenile' specimens were identified based on the presence of physiological deciduous or mixed dentition (Winer *et al.*, 2013) and were disqualified from further study.

The teeth present, the surrounding alveolar bone and the TMJs were inspected systematically according to predefined criteria (Table 1) utilized in former studies (Verstraete *et al.*, 1996a, b; Abbott and Verstraete, 2005; Arzi *et al.*, 2013a, b). Special care was applied to rule out post-mortem trauma or preparation artefacts such as hairline cracks, artefactual sharp-edged fractures and flaked off enamel or artefactual tooth loss potentially caused by excessive heating and/or drying during skull preparation.

The presence or absence (i.e. congenital, acquired or artefactual) of all teeth was recorded. Empty alveoli with sharply delineated edges were considered to reflect post-mortem tooth loss during skull preparation and were noted as such. The number of teeth present was used to calculate the prevalence of abnormally formed teeth, attrition/abrasion, dental fractures and enamel hypoplasia; a full set of dentition was assumed when calculating prevalence of supernumerary teeth, bony changes consistent with periodontitis and periapical lesions.

All teeth were assessed for normal or abnormal form. The number of roots was determined primarily by assessing the visible part of the coronal root. If the tooth could be removed from the alveolus, the roots were examined or the alveoli themselves were examined when a tooth was artefactually absent. The skulls were examined for presence of supernumerary teeth adjacent to normal teeth, as well as for the presence of any persistent deciduous teeth. Teeth were evaluated for signs of attrition and/or abrasion (loss of enamel ± dentine due to wear) and severity was

Table 1
Congenital, developmental and acquired abnormalities noted and inclusion criteria

Observation	*Criteria*
Tooth absent artefactually	Jaw fragment missing or tooth absent, but a well-defined, sharp-edged, normally shaped, empty alveolus present; tooth presumed lost during preparation or post-mortem manipulation of the skull.
Tooth absent − presumably acquired	Tooth absent; alveolus or remnant alveolus visible; alveolar bone shows pathological signs (i.e. rounding of the alveolar margin, shallow alveolus, periosteal reaction on alveolar bone, increased vascular foramina).
Tooth absent − presumably congenital	Tooth and alveolus absent; smooth, morphologically normal bone present at the site; no physical space for that tooth to have occupied.
Malformed tooth	Presence of an abnormally shaped crown.
Number of roots	The number of roots, inspected directly or inferred from an empty alveolus or from the portion of root(s) visible within the alveolus (if the tooth is glued in place).
Supernumerary tooth	Presence of a supernumerary tooth adjacent to an expected tooth (or alveolus).
Persistent deciduous tooth	A persistent deciduous tooth adjacent to an erupted or unerupted permanent tooth.
Attrition/abrasion stage 1	Normal/abnormal wear of enamel, without dentine exposure.
Attrition/abrasion stage 2	Normal/abnormal wear leading to exposure of dentine on a cuspal tip, without tertiary dentine formation.
Attrition/abrasion stage 2	Normal/abnormal wear leading to exposure of tertiary dentine.
Attrition/abrasion stage 3	Normal/abnormal wear leading to pulp exposure.
Enamel fracture	A chip fracture or crack of the enamel only.
Uncomplicated crown fracture	A fracture involving enamel and dentine, but not exposing the pulp.
Complicated crown fracture	A fracture involving enamel and dentine, with pulp exposure.
Uncomplicated crown−root fracture	A fracture involving enamel, dentine and cementum, but not exposing the pulp.
Complicated crown−root fracture	A fracture involving enamel, dentine and cementum, with pulp exposure.
Root fracture	A fracture affecting dentine, cementum and the pulp.
Periapical lesions	Macroscopically visible periapical bone loss, root tip resorption, sinus tract formation originating periapically or obvious focal periosteal reaction overlying the apex.
Periodontitis stage 2	Evidence of increased vascularity at the alveolar margin (more prominent vascular foramina in and slightly rougher texture of, the bone of the alveolar margin).
Periodontitis stage 3	Rounding of the alveolar margin; moderate horizontal or vertical bone loss.
Periodontitis stage 4	Widening of the periodontal space; severe horizontal or vertical bone loss; tooth mobile in the alveolus.
Fenestration/dehiscence	Lack of buccal/labial or palatal/lingual alveolar bone, which results in exposure of the root surface with or without involvement of the coronal margin of the alveolar bone.
Enamel hypoplasia	Irregular pitting or a band-shaped absence or thinning of the enamel
TMJ osteoarthritis grade 1	Evidence of early periarticular new bone formation/osteophytes with minimal or no subchondral bone change.
TMJ osteoarthritis grade 2	Periarticular new bone formation and/or subchondral bone changes.
TMJ osteoarthritis grade 3	All previously described signs are present and more pronounced; subchondral bone lysis present; partial or complete ankylosis may be observed.

recorded as mild (minor dentine exposure), moderate (exposure of tertiary dentine) or severe (pulp exposure). Six dental fracture types were assigned according to the World Health Organization classification of human dental fractures, as modified for use in carnivores (Verstraete, 2003). Periapical lesions were considered as a bony fenestration or a periosteal reaction overlying the apex of a tooth. Periodontal status was assessed based on an established classification system adapted for use on dry skulls (Verstraete *et al.*, 1996a, b). Periodontitis stages 2−4 were assigned to the bony lesions indicative of periodontitis; periodontal disease stage 1 was excluded, as it refers to gingivitis, a soft tissue lesion that cannot be assessed on skull specimens. Stage 2 shows increased vascularity (i.e. increased bone porosity) at the alveolar margin. Stage 3 shows rounding of the alveolar margin with more than 3 mm of vertical or horizontal bone loss with furcation involvement. Periodontitis stage 4 shows widening of the alveolar margin with severe vertical or horizontal bone loss and teeth that are unstable in the alveoli or missing secondary to alveolar changes and/or furcation exposure. Since fenestration or dehiscence of the alveolar bone can be attributed to both anatomical developmental changes as well as periodontitis, this lesion type was evaluated separately. Enamel changes consistent with the clinical signs of enamel hypoplasia were also recorded.

When examining the TMJs, the mandibular heads on the condylar process and mandibular fossae of the squamous temporal bone were inspected independently. A semi-quantitative scoring system (Arzi *et al.*, 2013a, b) for osteoarthritis (OA) was applied to each affected bone. Stages of TMJ-OA were scored as mild (early periarticular new bone formation/

osteophytes with minimal or no subchondral bone change or for increased porosity or irregular texture of the articular surface[s]), moderate (more pronounced periarticular new bone formation and/or subchondral bone destruction) or severe (severe periarticular new bone formation/osteophytes, marked subchondral bone destruction or partial or complete ankylosis).

Data from all adult and young adult specimens were pooled according to tooth type and were analysed with descriptive statistics. Prevalence of lesions was compared with age group and sex using Fisher's exact test. $P < 0.05$ was considered significant.

Results

The collection date of the examined skulls ranged from 1960 to 2015. Of the 392 specimens examined, 185 neonatal and juvenile skulls with deciduous or mixed dentition, specimens with excessive artefactual tooth loss or trauma, as well as incomplete skulls (i.e. missing mandible[s] or maxilla[e]) were excluded from further study. The 207 remaining skulls included 124 skulls (59.9%) of young adults and 83 (40.1%) of adults. Sixty-five (31.4%) were from male wolves, 104 (50.2%) were from females and for 38 (18.4%) the sex was unknown. None of the pathological changes evaluated had a sex predilection. Age predilection is reported when statistically significant.

Presence of Teeth

Only 72 specimens (34.8%) had all teeth present. Out of the 136 skulls (65.4%) with tooth loss, 81 (59.6%) were young adults and 55 (40.4%) were adults. Forty-four (32.4%) males and 74 (54.4%) females had missing teeth.

Thirty of the skulls (14.5%) were noted to have congenitally missing teeth and 19 wolves (9.2%) experienced tooth loss during their life, while 109 skulls (52.7%) were artefactually missing teeth (Fig. 1). Out of 8,694 possible teeth, 8,339 (95.9%) were present for evaluation. Of the 355 missing teeth, 55 (15.5%) were absent congenitally, 30 (8.5%) were lost during life and 270 teeth (76.1%) were artefactually lost *post mortem*. Of the 30 skulls with congenitally absent teeth, 14 (46.7%) were young adults and 16 (19.1%) were adults; nine (30%) were males and 12 (40%) were females. The teeth that were most commonly absent congenitally were maxillary and mandibular first premolar teeth ($n = 34$, 61.8%), followed by mandibular third molar teeth ($n = 10$, 18.2%). Acquired tooth loss was proportionally significantly more common in adults ($n = 13$, 15.5%) than in young adults ($n = 6$, 4.8%; $P = 0.013$). Acquired tooth loss was found predominantly among premolar teeth of both the maxillae and mandibles ($n = 16$, 53.3%). Artefactual tooth loss was represented highest among maxillary first premolar teeth ($n = 81$, 30.0%) (Fig. 2) followed by mandibular third molar teeth ($n = 62$, 23.0%).

Malocclusion

Malocclusion of skeletal or dental origin was noted in 37 skulls (17.9%), represented by 21 (56.8%) young adults, 16 (43.2%) adults, 18 (48.6%) males and 13 (35.1%) females. The most common form of skeletal malocclusion was presence of a level bite ($n = 26$, 70.3%, Fig. 3), as opposed to the normal incisor scissor bite. Crowding of the mandibular or maxillary premolar teeth was noticed in six specimens (2.9%, Figs. 4A and B), three of which were also affected by a level bite, while a rostral cross-bite was present in four (1.9%). Another six specimens (2.9%) had a combined total of 10 teeth affected by dental malocclusion. Three skulls were affected by bilaterally rotated maxillary third premolar teeth (Fig. 4D), with one of these skulls affected by dental malocclusion only. Both other skulls had crowding of the premolar teeth and one was additionally affected by a level bite. One specimen had a mesioverted left maxillary third premolar tooth, secondary to linguoversion of the ipsilateral mandibular canine tooth. Another specimen had a distoverted right maxillary first incisor tooth, while a third specimen had a palatoverted left maxillary first incisor tooth.

Supernumerary Teeth and Odontodysplasia

Supernumerary teeth or teeth affected by odontodysplasia did not occur in any of the specimens.

Enamel Hypoplasia

Enamel hypoplasia occurred in five skulls (2.4%) and eight teeth (0.1%) in total.

Abnormal Number of Roots

An abnormal number of roots was found on 13 skulls (6.3%), affecting 23 teeth (0.3%). There were 19 maxillary third premolar teeth and four mandibular fourth premolar teeth, each having a supernumerary root (Fig. 5A). Three-rooted maxillary third premolar teeth were noted bilaterally in nine skulls. Two specimens had a single-rooted mandibular fourth premolar tooth (Fig. 5B).

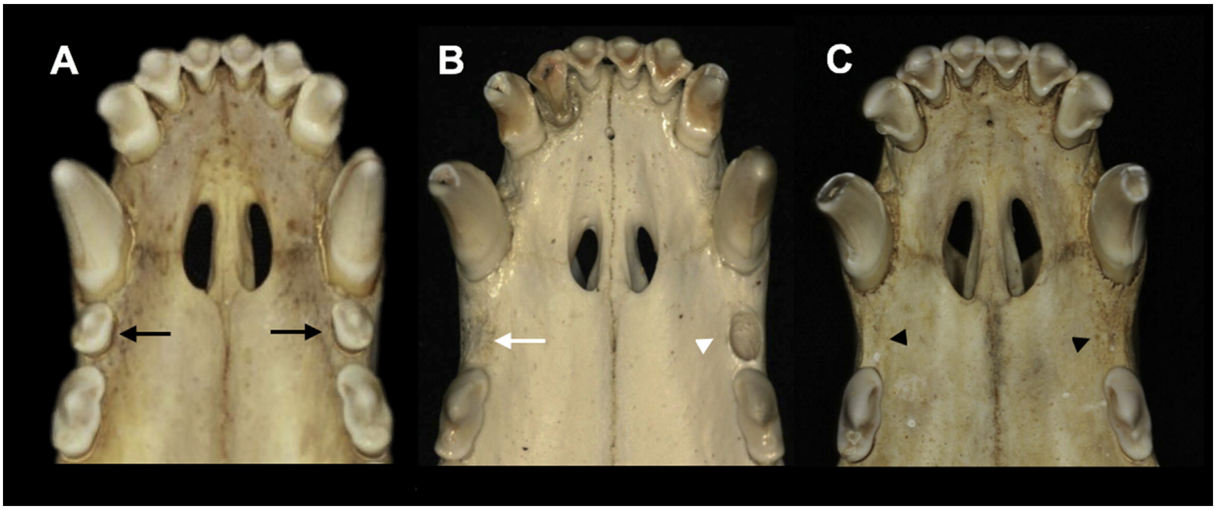

Fig. 2. Normal rostral maxillae (A, UAM 101238) with both maxillary first premolar teeth present (black arrows), compared with another specimen (B, UAM 47421) with acquired (white arrow) and artefactual (white arrowhead) maxillary first premolar teeth missing and a third skull (C, UAM 47188) with both maxillary first premolar teeth absent congenitally (black arrowheads). Refer to Table 1 for a description of criteria for absent teeth.

Persistent Deciduous Teeth

One persistent deciduous tooth was present in two skulls (1.0%, Fig. 5C), a deciduous right mandibular second premolar tooth and a left mandibular fourth premolar tooth.

Dehiscence and Fenestration

Out of the 8,339 teeth present, 203 teeth (2.4%) in 72 skulls (34.8%) were associated with lesions consistent with either dehiscence ($n = 183$, Fig. 6B) or fenestration ($n = 14$, Fig. 6C) or a combined lesion ($n = 6$). The teeth affected most commonly were the maxillary first molar teeth ($n = 104$, 51.2%), followed by the

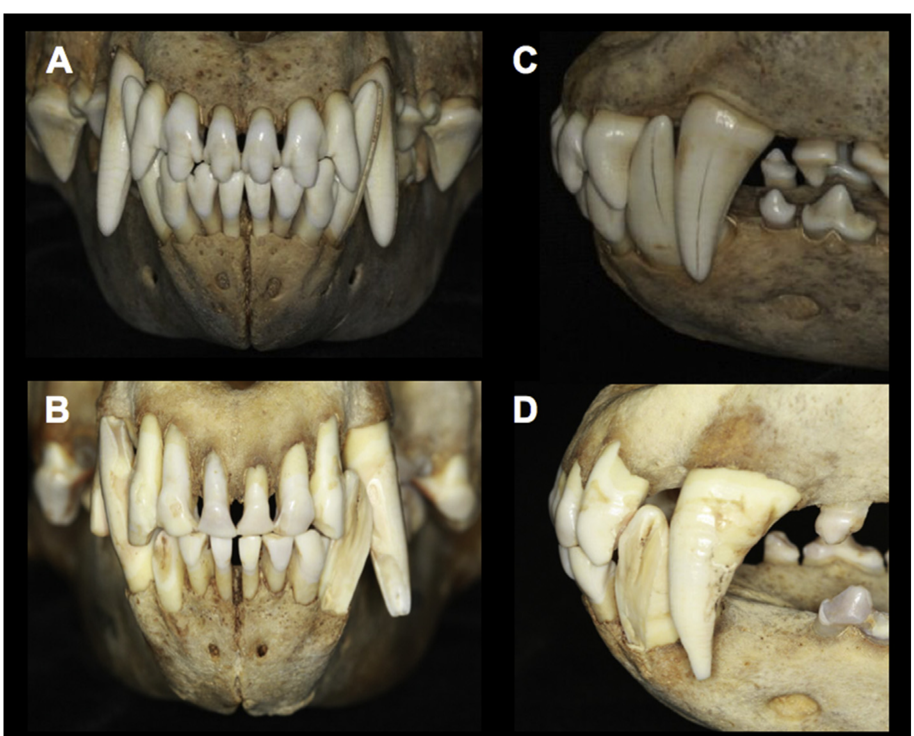

Fig. 3. Normal incisor and canine teeth occlusion (A and C, UAM 101238) and level bite (B and D, UAM 87911) in a grey wolf.

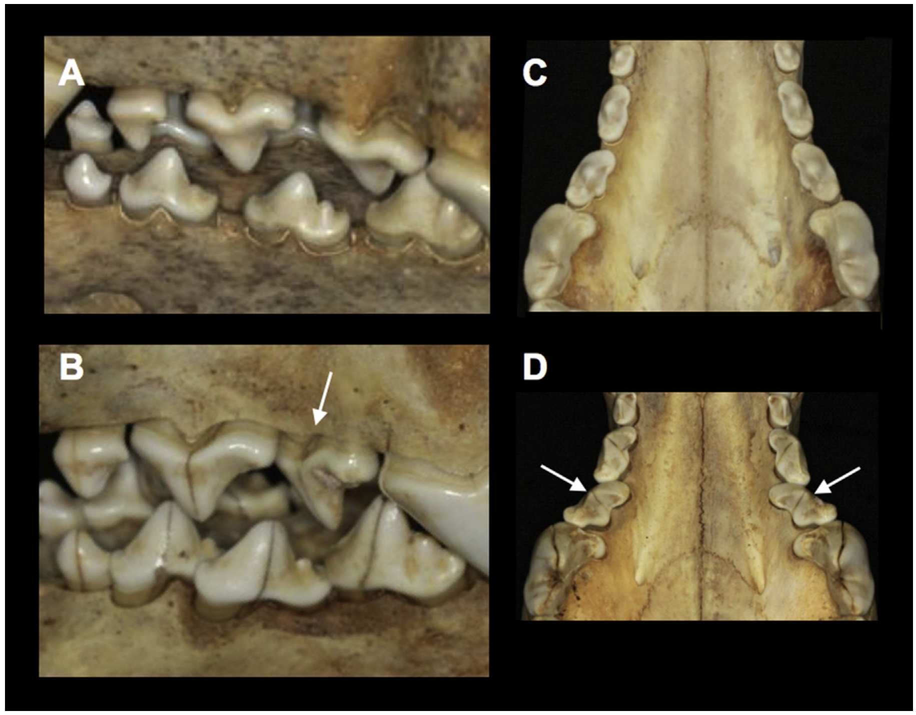

Fig. 4. Normal spacing (A and C, UAM 101238) compared with crowding (B and D, UAM 101238) of the premolar teeth, with bilaterally rotated maxillary third premolar teeth (B and D, arrows).

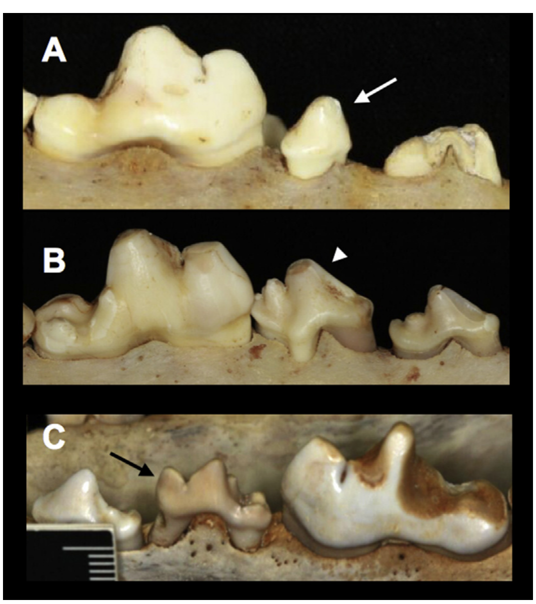

Fig. 5. (A) Single-rooted mandibular fourth premolar tooth (white arrow, UAM 47185), (B) 3-rooted mandibular fourth premolar tooth (white arrowhead, UAM 47460) and (C) persistent deciduous fourth premolar tooth (black arrow, UAM24108) in three different specimens.

maxillary second molar teeth ($n = 51$, 25.1%) and the maxillary fourth premolar teeth ($n = 48$, 23.7%). There was no predilection based on age or sex.

Periodontitis

Evidence of periodontal disease stages 2–4 was noted on 115 skulls (55.6%). Significantly more adults ($n = 62$, 74.70%, Fig. 6) than young adults ($n = 52$, 41.93%; $P < 0.0001$) showed signs of periodontal disease, while there was no significant difference in proportion among the affected 40 males (61.5%) and 56 females (53.3%). One thousand teeth (11.5%) were affected by periodontal disease stages 2–4. Incisor teeth were the most common to show signs of attachment loss ($n = 756$, 75.6%, Fig. 7), followed by premolar teeth ($n = 126$, 12.6%, Fig. 8) and canine teeth ($n = 64$, 6.4%). Stage 2 periodontal disease was seen most often, present on 865 teeth of 29 skulls (10.0% and 14.0%, respectively); stage 3 was noted on 107 teeth on 29 skulls (1.2% and 14.0%, respectively); only 28 teeth on 16 skulls (0.3% and 7.7%, respectively) were affected by stage 4 periodontal disease.

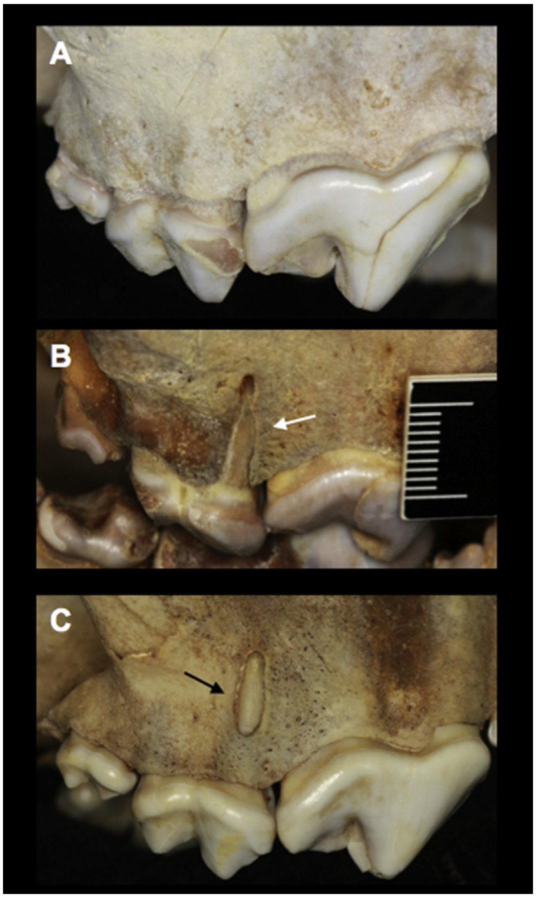

Fig. 6. (A) Normal anatomy of the alveolar bone margin (UAM 102025), compared with (B) a specimen with dehiscence (white arrow, UAM 92623) and (C) another specimen with fenestration (black arrow, UAM 49370) over the mesiobuccal root of the right maxillary first molar tooth. Bar, 1 cm. Refer to Table 1 for a description of criteria for dehiscence and fenestration.

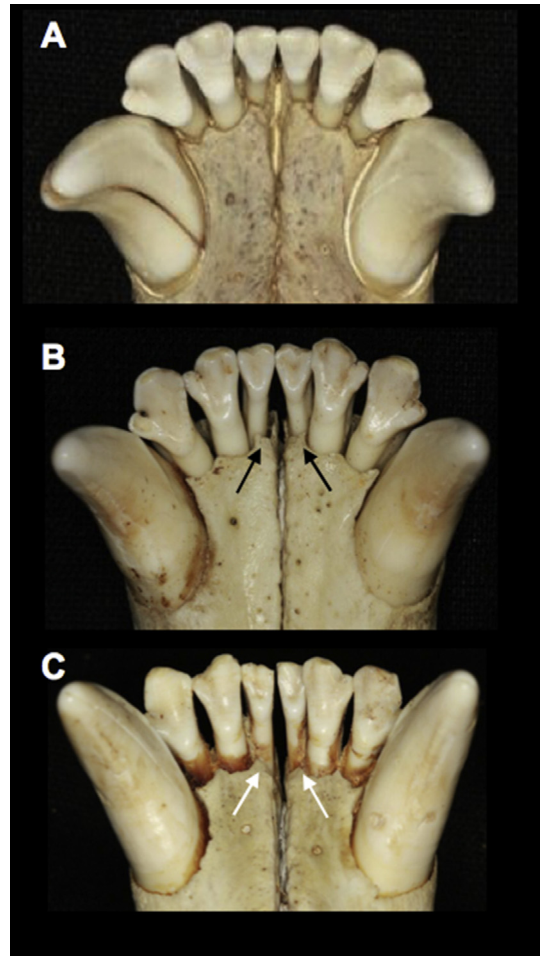

Fig. 7. (A) The mandibular incisor teeth with no sign of periodontitis (UAM 101238), in comparison with (B) periodontitis stage 2 (black arrows, UAM 21343) and (C) periodontitis stage 3 of the first mandibular incisor teeth (white arrows, UAM 21220). Refer to Table 1 for a description of the stages of periodontitis.

Endodontal Disease

One hundred and sixty-one skulls (77.8%) showed signs of endodontal disease, including attrition/abrasion (n = 144, 69.6%), fractured teeth (n = 103, 49.8%), periapical disease (n = 5, 2.4%) or caries (n = 1, 0.5%).

Attrition or abrasion was noted on 2,522 teeth (30.2%) in 144 skulls (69.6%), affecting significantly more adults (n = 70, 83.3%) than young adults (n = 74, 59.7%; P = 0.0004). Teeth most often affected by attrition/abrasion were: the premolar teeth (n = 811, 33.5%), with 434 maxillary (36.8%) and 377 mandibular (30.4%) premolar teeth affected; followed by molar teeth (n = 803, 51.9%), with 371 maxillary (57.3%) and 432 mandibular (48.0%) molar teeth affected; incisor teeth (n = 762, 40.2%), with 416 maxillary (43.5%) and 346 mandibular (36.7%) incisor teeth affected; and 146 canine teeth (23.6%), with 85 maxillary (28.1%) and 61 (19.2%) mandibular canine teeth affected (Fig. 9). Mild abrasion was found on 723 teeth (8.7%) in 96 skulls (46.4%), most commonly affecting the maxillary (n = 145, 15.2%) and mandibular (n = 125, 13.3%) incisor teeth. Moderate abrasion was noted on 1,384 teeth (16.6%), with the molar teeth (n = 455, 29.4%) being most affected, divided between 207 maxillary (32.0%) and 248 mandibular (27.5%) molar teeth. Sixty-three teeth (0.8%) on 28 skulls (13.5%) were affected by severe abrasion. This most severe form of wear mainly affected the molar (n = 33, 0.4%) and premolar teeth (n = 25, 0.3%); specifically, 15 maxillary first molar teeth, 15 mandibular first molar teeth and 14 maxillary fourth premolar teeth.

Fractures were noted on 424 teeth (5.1%) of 103 skulls (49.8%), with significantly more adults

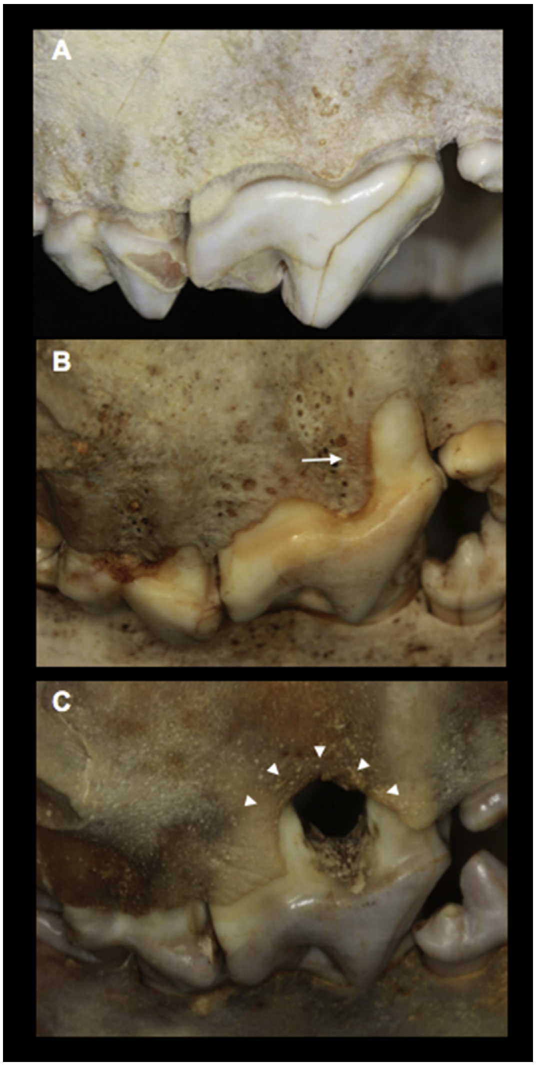

Fig. 8. Comparison of (A) a healthy alveolar bone (UAM 102025), (B) periodontitis stage 3 (white arrow, UAM 49838) and (C) periodontitis stage 4 (white arrow heads, UAM 101203) of a right maxillary fourth premolar tooth. Refer to Table 1 for a description of the stages of periodontitis.

($n = 60$, 71.4%) than young adults ($n = 43$, 34.7%) affected (Fig. 10). Of all fractured teeth, complicated crown fractures ($n = 140$, 33.0%) were most common, followed by complicated crown–root fractures ($n = 125$, 29.5%), root fractures ($n = 124$, 29.3%), uncomplicated crown fractures ($n = 21$, 5.0%), uncomplicated crown–root fractures ($n = 10$, 2.4%) and enamel fractures ($n = 4$, 0.9%). Based on the number of corresponding teeth present, canine teeth were most often affected by tooth fractures ($n = 56$, 7.0%), compared with premolar teeth ($n = 191$, 6.00%), incisor teeth ($n = 121$, 5.0%) and molar teeth ($n = 56$, 2.8%).

Periapical disease was associated with only six teeth (0.1%), found on five (2.4%) skulls. One tooth (0.01%) on one specimen (0.5%) was noted to have a mild carious lesion without pulp exposure.

Temporomandibular Joint Disease

Lesions consistent with temporomandibular joint osteoarthritis (TMJ-OA) were found in 24 skulls (11.6%), involving 38 joints (9.2%). The mandibular fossa was affected in only one specimen and all other lesions were present at the mandibular head. Fourteen skulls had bilateral TMJ-OA. The right mandibular head was affected in 20 (Fig. 11) and the left in 18 skulls. Lesions were located centrally on the mandibular head in 19 TMJs, the lateral aspect of the mandibular head was affected in 11, the medial aspect was affected in four and the entire joint surface was affected in five wolves. The one diseased fossa was affected over its entire joint surface. Four mandibular heads exhibited osteochondritis dissecans (OCD)-like lesions. Mild TMJ-OA accounted for 60.5% ($n = 23$), moderate TMJ-OA or 15.8% ($n = 6$) and severe TMJ-OA was present in 23.7% ($n = 9$) of the diseased joints.

Trauma

Maxillofacial trauma was noted in 44 skulls (21.3%), comprising 25 skulls with bullet wounds, 17 specimens with bite marks, one high-impact blunt trauma and one skull with signs of both bite marks and bullet wounds (Fig. 12).

Discussion

The prevalence of anatomical or developmental lesions, such as congenitally missing teeth, malocclusion or enamel hypoplasia, was fairly low in the examined population of Alaskan wolves, as opposed to more commonly encountered acquired lesions such as attrition/abrasion, dental fractures or periodontitis. Other lesions, such as an abnormal number of roots, fenestration and dehiscence or TMJ-OA, have not been reported in grey wolves before.

Ante-mortem tooth loss has been reported previously in 17–29% of wolves across their habitat (Van Valkenburgh, 1988), affecting only about 0.9% of teeth (Losey *et al.*, 2014). In this study, ante-mortem tooth loss was further classified into congenital and acquired loss, with 14.5% and 9.2%, respectively. The most common congenitally missing teeth, the maxillary and mandibular first premolar teeth and the mandibular third molar tooth, are the first teeth to disappear in the dental formula from an evolutionary standpoint. It is not clear if there is a genetic drift towards a decreased number of teeth in the grey wolf. However, hypodontia, affecting the

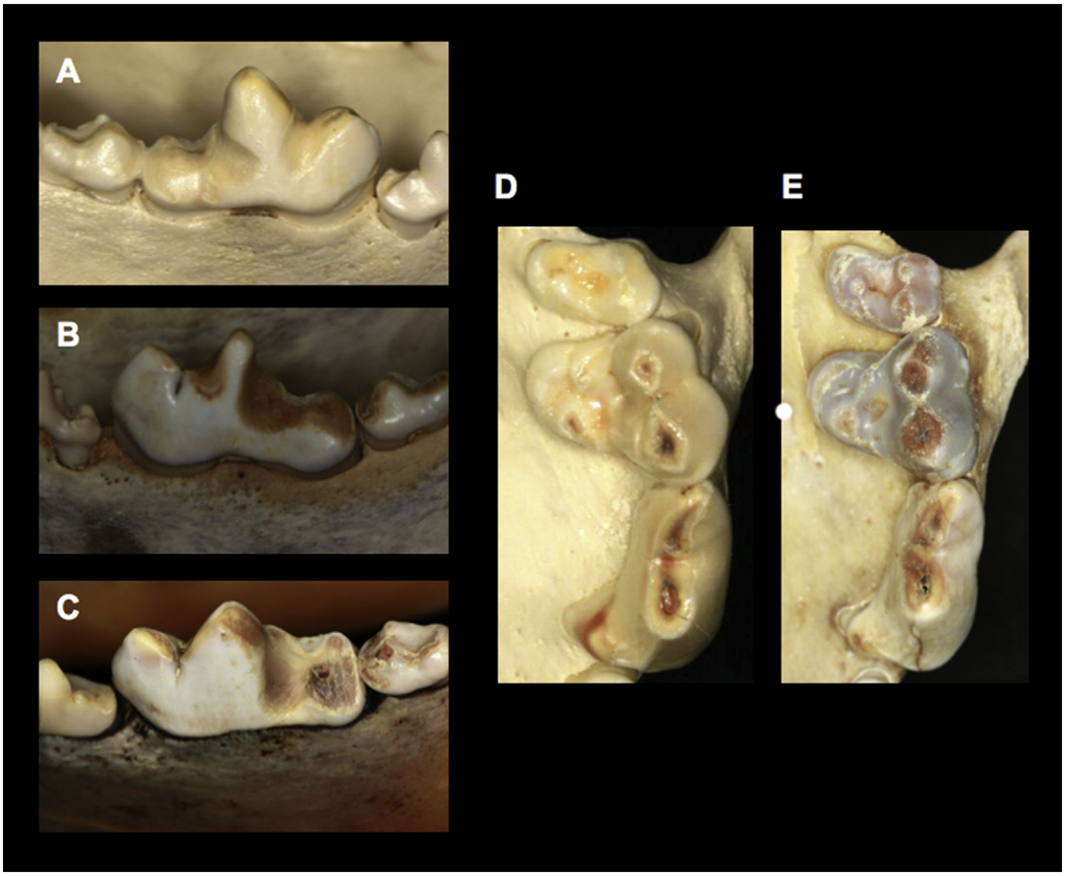

Fig. 9. Different specimens with signs of attrition/abrasion: (A) stage 2 of a right mandibular first molar tooth (UAM 100504), (B) stage 3 of a left mandibular first molar tooth (UAM 24108), (C) stage 4 of a left mandibular first molar tooth (UAM 21244), (D) stage 1 on a right maxillary second molar tooth and stage 3 on a maxillary first molar and fourth premolar tooth (UAM 70584) and (E) stage 2 on a right maxillary second molar tooth, stage 3 on a maxillary first molar tooth and stage 4 on a right maxillary fourth premolar tooth (UAM 101217). Refer to Table 1 for a description of the stages of attrition/abrasion.

first premolar teeth, has been reported previously to be associated with an autosomal recessive genetic abnormality in small domestic animals, including the domestic dog (Harvey and Orr, 1990).

In a study of 500 wolves from the former Soviet Union and Europe, only 3% showed malocclusion, while in 131 wolves of a heavily inbred Scandinavian population 13.7% exhibited some kind of skeletal malocclusion, including four wolves with mandibular brachygnathia, which had never been reported before (Vilà *et al.*, 1993; Räikkönen *et al.*, 2013). In this study, we reported malocclusion of skeletal or dental origin in 17.9% of skulls, most of which were mild forms not leading to hard tissue trauma or other bony abnormalities. Severe forms of malocclusion, as reported in the inbred population in Scandinavia, were not found in any of the examined Alaskan wolf skulls.

Dehiscence is an alveolar bone defect, which results in exposure of the root surface at the cervical region, involving the coronal margin of the alveolar bone (Davies *et al.*, 1974). When the coronal margin of the alveolar bone is still intact and only an isolated area of the root is lacking bone coverage, the defect is termed fenestration (Enhos *et al.*, 2012; Yagci *et al.*, 2012; Buyuk *et al.*, 2016). Dehiscence and fenestration of the alveolar bone can either be an anatomical/developmental variation or be caused by periodontal or endodontal disease. In human dentistry, the periodontium is considered healthy when the crest of the interproximal bone is positioned within 2 mm apical to the cementoenamel junction (CEJ). Although dehiscence and fenestration have been noted in recent studies of a variety of wild mammals, such as polar bears, walruses or brown bears (Winer *et al.*, 2016a, b, 2017), this study is the first to note fenestration and dehiscence in grey wolves, with 2.4% of the present teeth displaying one or both of these lesions. The teeth most commonly affected, the maxillary fourth premolar teeth and both maxillary molar teeth, are situated at the caudolateral aspect of the maxilla, with the buccal roots usually covered by

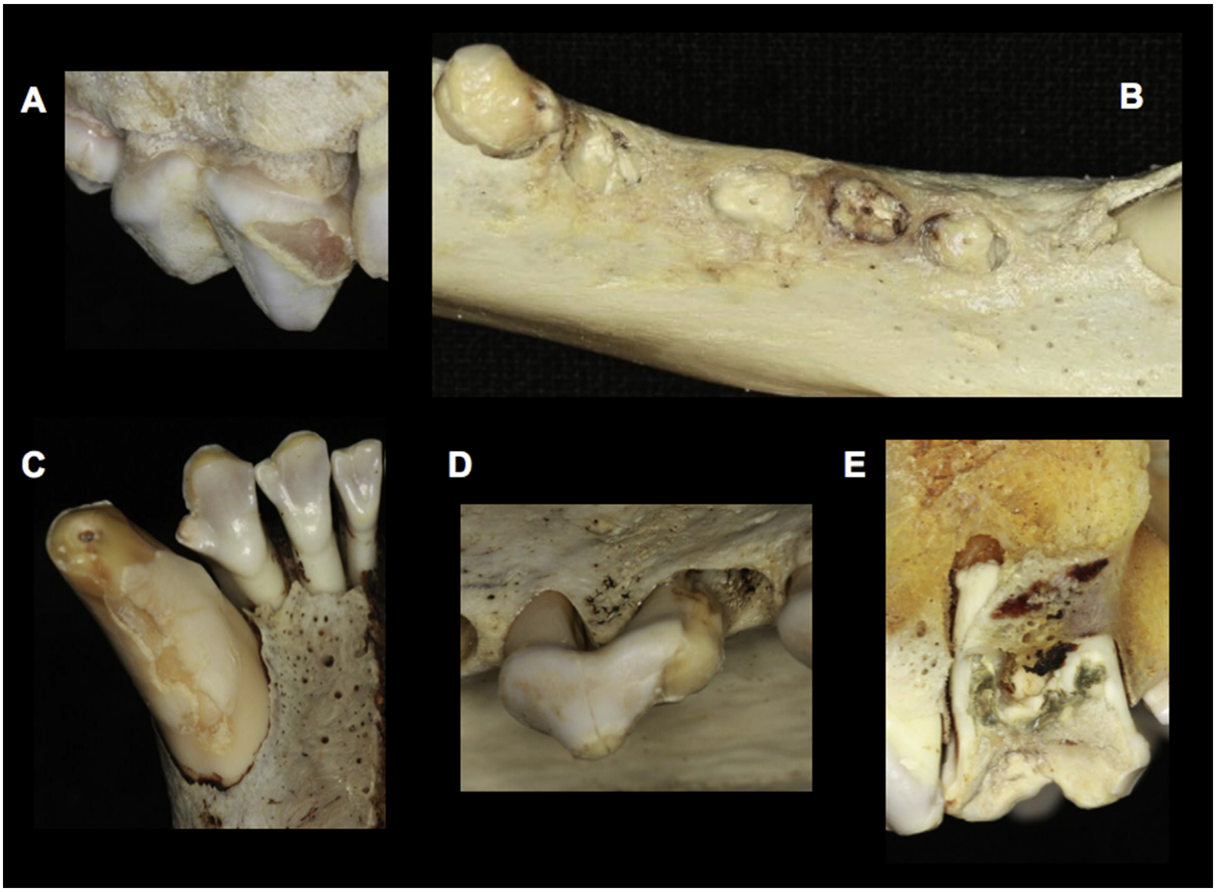

Fig. 10. Examples of different types of tooth fractures: (A) enamel fracture of a right maxillary first molar tooth (UAM 102025), (B) root fractures of the left mandibular first, second and third premolar tooth (UAM 100422), (C) complicated crown fracture of the left mandibular canine tooth (UAM 21235), (D) uncomplicated crown—root fracture of the left maxillary second premolar tooth with signs of external inflammatory root resorption (UAM 21361) and (E) complicated crown—root fracture of the left maxillary first molar tooth with a periapical lesion (UAM 21235). Refer to Table 1 for a description of the different types of tooth fractures.

only a thin plate of alveolar bone, which may thus be predisposed to bone loss.

Although periodontal disease includes soft tissue inflammation, which cannot be evaluated on dry skulls, the associated hyperaemia leads to an increase of vascular porosity and rough texture of the bone along the alveolar margin, which can be noted on post-mortem bony examination. Additionally, horizontal and vertical alveolar bone loss can be assessed, which further helps to characterize the severity of periodontal disease *post mortem*. While periodontal disease is one of the most common diseases in domestic dogs (Harvey, 1998), affecting 80% of dogs over 4 years of age, it has only been described in 8.8% of Croatian grey wolves (Pavlović *et al.*, 2007) and 12.4% of wolves in the former Soviet Union and neighbouring countries (Vilà *et al.*, 1993). In the present study, over half of the skulls were noted to have one or more teeth affected by periodontitis. Since the populations in both Croatia and the former Soviet Union are smaller than in Alaska, it is unlikely that genetic predisposition is responsible for this substantial difference in prevalence. It is possible that the occurrence of periodontitis has been inadvertently inflated in this study by scoring a variation of normal as a form of pathology or that more subtle stages were noted. As periodontitis is a progressive disease without oral care intervention, it was not surprising to find significantly more adults affected than younger individuals.

Endodontal disease, such as abnormal wear or tooth fractures, can become acutely painful when the dentinal tubules and pulp are exposed. Furthermore, disruption of the integrity of the crown and/or root can affect the function of that tooth and therefore the functionality of the dentition as a whole. In a previous study, all wolves included showed some stage of wear, with males being affected more severely than females (Van Valkenburgh, 1988). The present study noted tooth wear in over two-thirds of the examined skulls; however, a significant sex predilection was not found. Hunting large prey, inter- and intraspecies

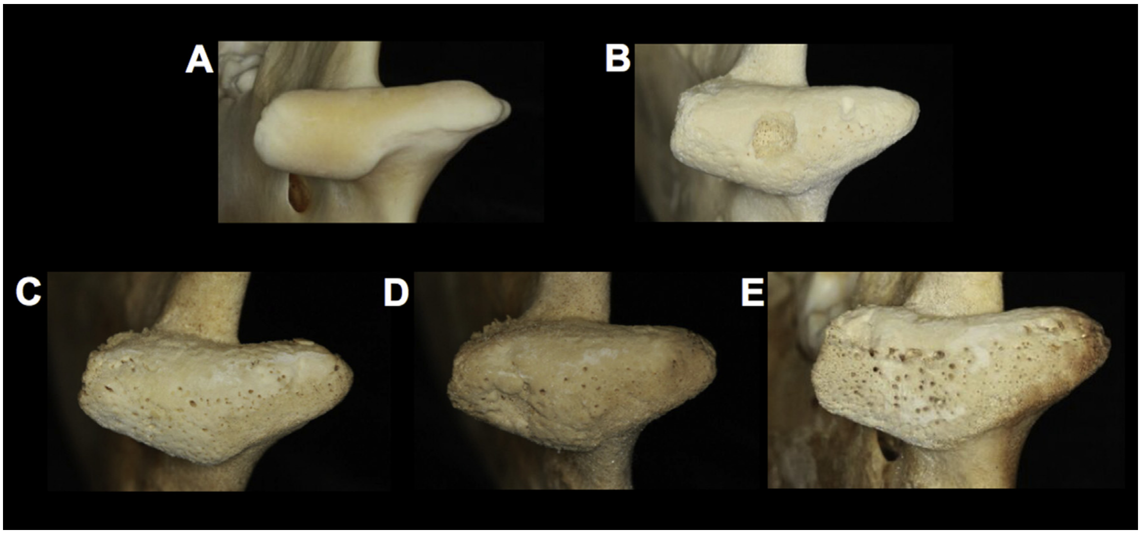

Fig. 11. Comparison of (A) a healthy right mandibular head (UAM 115902) with diseased right mandibular heads displaying (B) a central OCD-like lesion grade 3 (UAM 70583) and (C) OA grade 1 (UAM 87890), (D) OA grade 2 (UAM 87884) and (E) OA grade 3 of the entire mandibular head (UAM 102040). Refer to Table 1 for a description of the different grades of TMJ-OA.

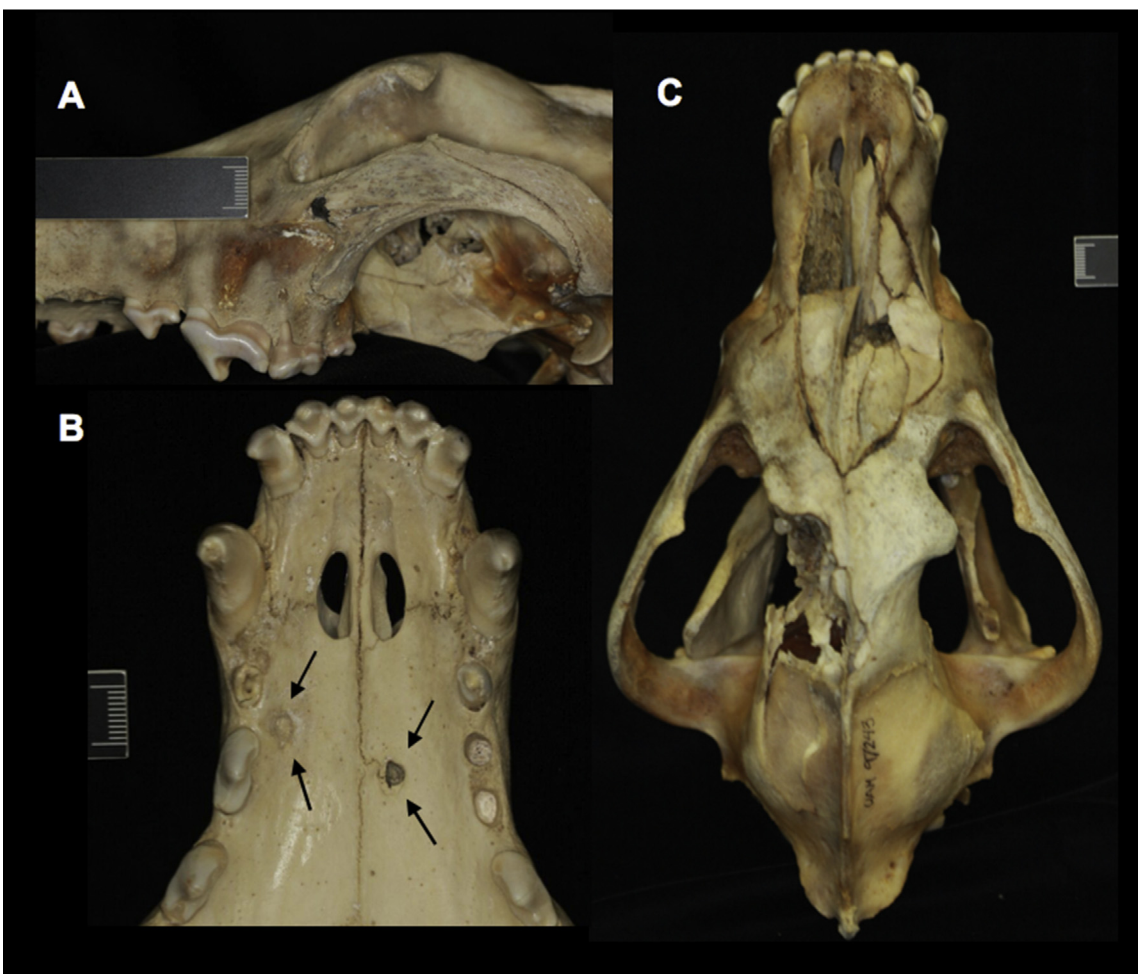

Fig. 12. Different examples of trauma: (A) a bullet incorporated into the left zygomatic arch (UAM 97243), (B) evidence of bite wounds to the palate with evidence of healing (arrows, UAM 101258) and (C) severe, blunt trauma to the maxillofacial region (UAM 97243). Bar, 1 cm.

altercations and mastication of bone can all lead to traumatic damage of teeth (Van Valkenburgh, 1988; Vilà *et al.*, 1993; Leonard *et al.*, 2007; Losey *et al.*, 2014). Nearly half of the studied skulls exhibited fractured teeth, as opposed to a previous study noting tooth fractures in only 27.8% of specimens (Losey *et al.*, 2014). Another study noted that premolar and canine teeth were most often affected by fractures (Van Valkenburgh, 1988), which is in agreement with the findings of the present study.

Abnormal wear of teeth and incidence of tooth fractures appear to be correlated positively with each other, as well as with increasing age. In the present study only 10% of grey wolves with mild wear also possessed one or more fractured teeth, while 40% of wolves with moderate and 90% of wolves with severe wear exhibited one or more tooth fractures. Another study concluded that severe tooth abrasion with exposure of tertiary dentine was mostly seen on the contralateral side of severe and likely painful lesions, such as fractures or severe stages of periodontitis (Pavlović *et al.*, 2007). While it does appear plausible that painful lesions on one side of the mouth may lead to the increased use of the contralateral side, we did not evaluate the skulls for lesion severity per side.

Periapical lucencies are commonly associated with fractured teeth and severe periodontitis (Lommer and Verstraete, 2000). In this study, out of the six teeth associated with periapical bone loss, the periapical lesion of all four affected premolar teeth was associated with a complicated crown fracture, while the periapical lesion of the two affected molar teeth was likely caused by the concurrently present moderate to severe abrasion.

TMJ disorders may be accompanied by signs of pain and decreased function of the joint (Tanaka *et al.*, 2008). In the present study, only an eighth of the wolves had evidence of TMJ disease, with the majority having OCD-like lesions. It is impossible to know whether the wolves were affected clinically by their TMJ disorders. A study examining the TMJ of dogs with the use of computed tomography concluded that 30% of the evaluated dogs had lesions consistent with TMJ disorders, most commonly TMJ-OA, and further noted that not all dogs affected also displayed clinical signs of TMJ disease (Arzi *et al.*, 2013a). While in dogs the medial aspect of the joint was more often involved, in wolves the lesions were mostly centrally located on the mandibular head. Although we cannot conclude why these wolves were affected by TMJ-OA, possible causes include genetic factors, aberrant load on the TMJ, abnormal ossification, focal trauma, avascular necrosis or a combination thereof (Campos *et al.*, 2005; Tanaka *et al.*, 2008).

Multiple specimens had signs of bite marks and bullet wounds, some of which had signs of bone healing associated with those lesions, which would imply they did not die as a result of these injuries until at least some time had passed to allow for healing.

Overall, a wide range of dental and TMJ diseases and disorders were noted on the examined selection of grey wolf skulls. Compared with domestic dogs, wolves appear less prone to periodontal disease, which may be caused by a genetic predisposition for poorer periodontal health in domestic dogs; in contrast, wolves appear more prone to endodontal disease, consistent with the fact that they are more exposed to tooth damage during hunting, killing prey or altercation amongst wolves or different species.

The dentition is considered to play a major role in the survival of wolves and individuals affected by severe lesions, such as attrition/abrasion or tooth fracture with pulp exposure, severe periodontitis or periapical disease or marked TMJ disorders, likely suffered from discomfort and pain as a consequence. Whether these lesions contributed to the mortality of grey wolves in this population could not be deduced from the present data, as the majority of wolves in the collection did not die of natural causes, but were instead killed at a relatively young age. Further studies are necessary to evaluate the effect of dental disease on the life and health of wolves.

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Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jcpa.2018.03.001.

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