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1 The evolution of mechanisms involved in vertebrate endothermy

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10

11 **Abstract**

12 Endothermy, i.e. the endogenous production of metabolic heat, has evolved multiple times
13 among vertebrates, and several strategies of heat production have been studied extensively by
14 physiologists over the course of the 20th century. The independent acquisition of endothermy
15 by mammals and birds has been the subject of many hypotheses regarding their origin and
16 associated evolutionary constraints. Many groups of vertebrates, however, are thought to
17 possess other mechanisms of heat production, and alternate ways to regulate thermogenesis that
18 are not always considered in the palaeontological literature.

19 Here we perform a review of the mechanisms involved in heat production, with a focus
20 on cellular and molecular mechanisms, in a phylogenetic context encompassing the entire
21 vertebrate diversity. We show that endothermy in mammals and birds is not as well defined as
22 commonly assumed by evolutionary biologists, and consists of a vast array of physiological
23 strategies, many of which currently unknown. We also describe strategies found in other
24 vertebrates, which may not always be considered endothermy, but nonetheless correspond to a
25 process of active thermogenesis. We conclude that endothermy is a highly plastic character in
26 vertebrates, and provide a guideline on terminology and occurrences of the different types of
27 heat production in vertebrate evolution.

28

29 *Keywords:* vertebrates, physiology, endothermy, evolution, metabolic rate, palaeobiology

30

31 **1. Introduction**

32 Endothermy, i.e. the ability for an organism to generate body heat through a specifically
33 dedicated metabolic activity, is considered to have appeared at least twice in the evolution of
34 vertebrates, in the mammalian and avian lineages [1,2]. Other vertebrate lineages are generally
35 labelled as ectotherms (i.e. organisms relying on their external environment as a main source
36 of heat; see a review with detailed terminology for thermal physiology in [2]). Mammalian and
37 avian types of endothermy have both been characterized in many studies and reviews [3–6],
38 and physiological processes behind metabolic heat production have been extensively studied at
39 the molecular level [7–11]. These processes are energetically very costly, and mammalian and
40 avian basal metabolic rates (BMR, i.e. an estimation of metabolism corresponding to the
41 minimal consumption of oxygen per unit of body mass over time, measured in controlled
42 conditions, i.e. in an adult, post-absorptive, resting, non-reproductive animal [2,12–14]; a
43 closely related estimator, resting metabolic rate (RMR), which is BMR without control for post-
44 absorptive state, is also commonly used, especially for ectotherms [14,15]) are usually higher
45 than that of ectotherms of the same size by at least an order of magnitude [2,16]. However,
46 many particular cases of endothermy diverging from this ‘general model’ have been described
47 among birds and mammals. Other types of thermogenic abilities have also been identified in
48 many other vertebrate groups, some of which being also described as ‘endothermy’ in the
49 literature (see below). Furthermore, the evolutionary origins of such processes are not currently
50 well understood, and many hypotheses have been proposed over the last decades to describe
51 how and why endothermy might have first appeared, with a particular focus on the stem groups
52 of birds (e.g. non-avian dinosaurs [17,18]) and mammals (i.e. non-mammalian synapsids
53 [12,19]). Indeed, since heat is a by-product of all metabolic processes [2], many studies have
54 described members of these lineages as having been subject to various evolutionary constraints
55 that could have favored the selection of endothermy (see below). Such studies have also tried

56 to estimate BMR for fossil species in those groups, using various predictive modeling
57 techniques [20–23]. Most of these studies have focused on non-avian dinosaurs, with numerous
58 and sometimes contradictory results being proposed over the last decade regarding their
59 potential endothermy [24,25].

60 While dinosaurian endothermy is still a highly controversial subject in the
61 palaeontological community, little attention has been given to the exact physiological strategy
62 that might have been used by non-avian dinosaurs to generate body heat. Many studies have
63 discussed the physiological abilities of several dinosaur clades by comparing them to a few
64 extant endothermic/homeothermic vertebrate species [18,21,22,26–28], suggesting potential
65 analogies between them in terms of heat production strategy and constraints. Other fossil groups
66 among archosauromorphs, as well as many extinct marine reptile clades, have also been studied
67 in this context [22,29–33]. However, none of these studies has ever discussed extensively the
68 physiology of these extant species in the larger context of endothermy in the whole vertebrate
69 clade, and there is no exhaustive review of the different strategies used by endothermic
70 vertebrates that would allow for a clear comparison between them in the context of
71 palaeophysiological inference. Furthermore, it is often difficult to compare two different
72 palaeontological studies describing fossil species as ‘endothermic’, due to the lack of a standard
73 terminology to describe those different strategies for the whole of vertebrates, and most of these
74 studies do not explicitly mention the kind of endothermy they are referring to.

75 In this paper, we describe as clearly and concisely as possible all major types of vertebrate
76 endothermy identified so far, with a focus on physiological mechanisms at the cellular, tissue
77 and/or organ level, and exceptions to the most common strategy in a given clade (whether
78 ectothermic or endothermic) are also discussed. This description is made in a phylogenetic
79 context (Fig. 1), with each type of endothermy being associated with the lineage(s) it has been
80 described in, and independent acquisitions of a particular type through evolutionary

81 convergence being mentioned explicitly. We also provide a synthetic comparative table (Table
82 1) for all different types of endothermy, which summarizes all adaptations linked with each of
83 them using a homogeneous nomenclature, and can be used as a quick checklist to navigate
84 through each of them in a straightforward way – which may sometimes prove difficult for non-
85 physiologists. Such a review is thus not as exhaustive as previous works on the subject focusing
86 more specifically on some vertebrate groups (e.g. [2,7,10,34–38]), but rather intended as a
87 framework for palaeontologists and evolutionary biologists to discuss physiological hypotheses
88 regarding the origin of endothermy in a given clade in a more precise way.

89

90 **2. Mammalian endothermy as a physiological strategy is not as well known as commonly** 91 **described**

92 Heat production in mammals has been a major field of research in physiology since the early
93 20th century [39], and the differences in that regard between mammalian clades – monotremes
94 and therians (i.e. marsupials and placentals) – have been extensively studied [9,36,40–42]. For
95 this reason, the physiological strategy of eutherians to produce metabolic heat is probably the
96 best-known type of vertebrate endothermy [43,44]. In placental mammals (i.e. eutherians),
97 metabolic heat is produced in brown adipose tissue (BAT), a structure found only in that clade
98 [11,43] (Table 1), through the activity of UCP1 (Uncoupling Protein 1; Supplementary Note
99 1). This process is usually labelled mammalian non-shivering thermogenesis [9] (NST, i.e.
100 endogenous heat production outside the thermoneutral zone without contraction of skeletal
101 muscles [43], as opposed to shivering thermogenesis (ST) – see section 5). NST allows most
102 eutherians to maintain their body temperature at a constant level (i.e. homeothermy), usually
103 within a range of 35–40°C [45], and is augmented in animals acclimated to the cold, a
104 phenomenon described as adaptive thermogenesis [43]. This adaptive NST is usually described

105 as the typical mammalian endothermy [9,11]; however, other strategies to produce metabolic
106 heat do exist among mammals, both in eutherians and non-eutherians.

107 Monotremes (i.e. the platypus *Ornithorhynchus anatinus*, and the four species of
108 echidnas), which do not possess BAT, display lower BMR and body temperatures (T_B) than
109 other mammals [46]. The platypus has the highest metabolic rate of all monotremes,
110 corresponding to about 70-80% of the corresponding eutherian values, whereas echidnas
111 present values of 25-40% of corresponding eutherian BMR, but all monotremes present
112 relatively similar T_B values – around 30–34°C depending on the ambient temperature (T_A)
113 [34,47]. Due to their wide range of T_B depending on T_A , very low BMR values, and ability to
114 enter daily torpor or hibernation (see below) in cold T_A with a drop in T_B of about 10°C
115 compared to its normal range, echidnas have often been characterized as ‘protoendotherms’,
116 i.e. reflecting an ancestral condition of thermoregulation that might provide insight on the
117 evolution of endothermy in placental mammals [34,35,47]. These low values of BMR and T_B ,
118 however, are indeed influenced by their phylogenetic position as monotremes, but also by their
119 burrowing lifestyle [48] and their diet, consisting almost exclusively of small arthropods [34].
120 Echidnas in particular are considered a very good example of the low metabolic performances
121 found in all myrmecophagous mammals compared to their close relatives with a different diet
122 [49]. Nevertheless, echidnas likely share some characteristics of early adaptations linked with
123 endothermy that might have been present in non-mammalian synapsids, such as moderately
124 high and highly labile BMR with facultative homeothermic endothermy (e.g. in reproductive
125 periods [34,35]), or strong insulation to prevent heat loss [19,35]. The platypus, conversely,
126 does not hibernate nor enter torpor [46], and is able to maintain its body temperature at a
127 constant level even when air or water temperature decreases below 10°C [40]. This might be
128 explained by the presence of local countercurrent exchange systems in its limbs, analogous to
129 that of tunas, lamnids sharks, and leatherback turtles (see description in section 4), ensuring a

130 high internal insulation to complement the integumental one provided by fur and subcutaneous
131 fat at low T_A [34,46]. Therefore, while it is possible that all monotremes share a similar heat
132 production mechanism, the platypus and echidnas have very different thermoregulation
133 strategies, the evolution of which is not yet understood [40].

134 Marsupials, by contrast, also have metabolic rate values lower than those of placentals,
135 but many of them are able to achieve a high level of thermoregulation despite their lack of BAT
136 [41,50]. The ability to display adaptive NST has been identified in several marsupial species,
137 but the mechanism involved is clearly different from that of placentals [41]. A high number of
138 mitochondria and high mitochondrial membrane surface density have long been known to be
139 important contributing factors to adaptive NST in eutherians [51]. It has been shown
140 experimentally, however, that marsupials present a higher mitochondrial proton leak than
141 eutherians of the same size, which is positively correlated with mass-specific BMR in both
142 eutherians and marsupials [50]. Differences in thermogenic abilities between eutherians and
143 marsupials are thus not linked with a difference in mitochondrial proton leak. The UCP1 gene
144 is present in monotremes and marsupials, but has been shown to be more similar in sequence
145 to its ectothermic (e.g. actinopterygians and lissamphibians) rather than eutherian orthologs,
146 and UCP1, if expressed at all, is thus unlikely to present a thermogenic function in those groups
147 [11,52]. Hence, while individual cases of NST in marsupials have been reported
148 (Supplementary Note 2), the exact mechanism underlying the endothermy of non-eutherian
149 mammals is currently unknown (Fig.1).

150 In fact, even among eutherians, the activation of UCP1 in BAT is not the only available
151 strategy to achieve NST. The *ucp1* coding sequence can be found in many other vertebrate
152 clades, including actinopterygians and amphibians, indicating that the gene likely appeared very
153 early in the evolution of vertebrates – at least in the last common ancestor of osteichthyans
154 [9,52]. The function of UCP1 orthologs in ectothermic vertebrates remains an enigma, and it is

155 widely accepted that UCP1 only acquired thermogenic abilities in the last common ancestor of
156 the eutherians, through its expression in BAT [41,52]. Several recent studies on the evolution
157 of the UCP1 gene in eutherians, however, have shown that inactivating mutations have
158 appeared in many large mammalian clades, which therefore lack the ability to express UCP1 in
159 their mitochondria to achieve NST (Supplementary Note 3). Further research is thus currently
160 needed to understand how alternative mechanisms to achieve NST have evolved independently
161 in placental mammals.

162 Apart from the regular homeothermic abilities found in most extant mammals and birds,
163 a large number of mammalian and avian species have developed abilities to punctually lower
164 their metabolic rates in response to specific environmental conditions (i.e. heterothermy: being
165 able to produce metabolic heat or not depending on the region of the body, or, like in this case,
166 over a certain period of time [2]). When exposed to cold weather during the night, sometimes
167 associated with food deprivation, several species in both clades will passively enter a state of
168 daily torpor, i.e. a drop in T_B to retain energy and decrease loss of body heat, usually associated
169 with lowering of metabolic rate [43] (Supplementary Note 4). Some species can also display
170 hibernation, i.e. longer, actively controlled periods of torpor for days or weeks, followed by an
171 arousal phase (Supplementary Note 4). Hence both passive and active control of features linked
172 with heat metabolism are ancestral to the mammalian and avian clades, and have been proposed
173 to have strongly influenced the rise of endothermy in non-mammalian synapsids and non-avian
174 dinosaurs [16,19]. Indeed, such a high plasticity in thermogenic metabolism provides the ability
175 to retain energy when needed by lowering BMR and T_B (e.g. during development, or when T_A
176 is high enough to ensure that T_B can be maintained at a relatively stable level through basking
177 behavior [16]), but also to achieve high metabolic performances to regulate incubation
178 temperature (the ‘parental care hypothesis’ [53]), or to forage at night when T_A is low to avoid
179 competition and/or predation from ectothermic vertebrates (the ‘nocturnal bottleneck

180 hypothesis' [54,55]). Departures from homeothermic endothermy in mammals and birds are
181 thus likely to be inherent to the acquisition of those features in the first place, and there is much
182 more than one single strategy to consider in order to understand the whole diversity and
183 evolution of the many physiological processes collectively referred to as mammalian
184 endothermy.

185

186 **3. Avian endothermy: the full extent of muscular thermogenesis**

187 Birds as a whole have higher metabolic rate than any other vertebrate clade, with BMR values
188 of a typical bird exceeding that of a typical ectotherm by a factor of about 15, compared to
189 about 12 for a typical mammal [2]. For that reason, their ability to achieve NST has been
190 extensively described in the literature [2,10,56]. The mechanism displayed by birds to produce
191 metabolic heat, however, is physiologically distinct from that of mammals: since BAT is a
192 synapomorphy of eutherians, it is absent in birds, and the gene coding for UCP1 was lost by
193 the whole reptile clade, so that birds do not use orthologs of mammalian UCPs to ensure NST
194 [57,58]. Another mitochondrial uncoupling protein, avian UCP (avUCP), has been described in
195 birds, predominantly expressed in skeletal muscles [59]. avUCP has multiple roles in avian
196 mitochondria, such as free-radical production or regulation of lipid use [59], and had originally
197 been hypothesized to have a role in heat production in birds, analogous to that of UCP1 in
198 mammals [60]. However, it has been experimentally shown not to have any role in
199 mitochondrial uncoupling activity nor heat production role in the muscles and liver in birds
200 [10]. Instead, the avian ortholog of the adenine nucleotide translocase (ANT), another protein
201 responsible for the transport of adenine nucleotides (i.e. ADP/ATP) through the inner
202 mitochondrial membrane, found in all eukaryotes [61], has been identified as the main protein
203 responsible for mitochondrial uncoupling in avian skeletal muscles. It has been hypothesized

204 to be directly involved in heat production in skeletal muscle thermogenesis [10,60]
205 (Supplementary Note 5).

206 However, even if mitochondrial ANT might play an important role in avian NST, heat
207 production in birds is likely to occur mostly through another specialized process, that originally
208 evolved from the muscular thermogenesis already present in all vertebrates: the production of
209 heat through uncoupling of calcium ions in the sarcoplasmic reticulum of muscle cells,
210 activated by sarcolipin (see also section 4 and Supplementary Note 6). In birds, this process
211 most likely became the main way to achieve NST through muscle hyperplasia, particularly that
212 of pectoral muscles, linked to the development of a large keeled sternum allowing muscle-
213 powered flight and a highly compact body plan [19,44,57]. In general, skeletal muscle mass is
214 much more developed in birds than in amniotes of the same size, and avian skeletal muscles
215 involved in flight have a high level of mitochondria and myoglobin, allowing a high aerobic
216 respiration capacity [36]. Avian muscle hyperplasia, likely driven by the ancestral acquisition
217 of bipedality in theropods and subsequent diversification of lifestyles in birds, is thus
218 considered to be a main factor for the emergence of both flapping flight and avian endothermy
219 [2,5,19,36], which could thus be characterized as evolutionary spandrels [62]. The evolution of
220 brooding behaviour and parental care has also been described as a potential factor for muscle
221 hyperplasia and the appearance of endothermy in several bird species [6,53]. Interestingly, the
222 loss of flight in birds also appears to be correlated with low values of BMR and T_B
223 (Supplementary Note 7). The lowest mass-independent BMR values known for birds can be
224 found in the New Zealand kiwis [63], with other flightless palaeognaths (e.g. emus and
225 ostriches) also showing low values compared to other birds [64], and the lowest avian T_B values
226 have been reported in flightless palaeognaths and penguins [45]. High BMR and flight
227 acquisition have also been suggested to be correlated with smaller genome size in birds and
228 mammals, albeit with some controversy [65–68].

229 Because avian endothermy is considered an exaptation of muscular hyperplasia linked
230 with the acquisition of bipedality in theropods, many evolutionary hypotheses have been
231 proposed over the past five decades regarding the endothermy of non-avian dinosaurs (Fig. 1),
232 especially since other characters previously associated with avian physiology have been
233 identified as present in the whole clade Dinosauria [69–73]. Many studies originally considered
234 dinosaurian metabolic rates to be low, assuming that their large size and inferred high insulation
235 were enough to ensure stable TB without metabolic heat production [17,26,74]. More recently,
236 high BMR values have been predicted by several studies for non-avian dinosaurs, particularly
237 large theropods [18,20–22]. This, however, does not necessarily mean that non-avian dinosaurs
238 achieved such high BMR by using the same physiological strategies as that of modern birds,
239 since similar BMR values can be achieved through many different processes, as shown in this
240 review. For this reason, the commonly inferred endothermy of non-avian dinosaurs cannot
241 currently be linked with any particular physiological strategy. Future studies focusing on
242 estimations of dinosaurian metabolic rates need to consider further palaeobiological features
243 (e.g. linked with pulmonary airflow [75], heart structure [76], lung structure [28], or nasal
244 conchae [77]; Table 1) in order to assess more precisely the actual physiological features that
245 might have given rise to such high metabolic rates in dinosaurs, whether closely related to those
246 of birds or not.

247

248 **4. Endothermy appeared at least six times in elasmobranchs and teleosts**

249 Mammals and birds are the classical model for endothermic vertebrates, and as such the
250 occurrence of thermogenic strategies in other clades is often overlooked. Indeed, several
251 lineages of elasmobranchs and teleosts independently developed specific forms of muscular
252 endothermy allowing them to elevate their body temperature relative to the ambient water
253 [38,78–81], with two main thermogenic strategies being found. In the first one, heat comes as

254 a byproduct from the contractions of the slow-twitch aerobic red muscles (RM) during constant
255 swimming, a configuration often called “red-muscle endothermy”. Specific anatomical
256 specialisations allow the region around RM to retain heat, including insulating them from the
257 outside cold water, and developing networks of counter-current blood vessels – called *retia* –
258 that keep blood warm by reducing convective heat transfer [38,82–85]. In the second one, called
259 “cranial endothermy”, extraocular muscles are transformed into a specialised heater organ, in
260 which muscular fibres have lost their contractile activity and perform futile calcium cycling
261 between the cytoplasm and the sarcoplasmic reticulum [7,86–88]. Thus, heater organs produce
262 a form of non-shivering thermogenesis (NST).

263 In elasmobranchs, mackerel sharks (Lamnidae) and the common thresher shark *Alopias*
264 *vulpinus* (Alopiidae) show elevated temperatures in their axial red muscles and in the brain
265 region [89–91]. In lamnids, the axial RM (part of the muscle blocks that surround the vertebral
266 column and supply most of the propulsive force for swimming) are located in a more medial
267 position than in closely related taxa, allowing them to be better insulated, and are associated
268 with *retia*. The brain region is heated by blood vessels carrying warm blood from the axial RM
269 [81–83]. Cranial and pectoral-fin *retia* have also been detected in several species of devil and
270 manta rays (Mobulidae), but their heat production strategy is so far unknown [92].

271 In teleosts, a very similar condition to that of lamnid sharks is found in tunas (Scombridae,
272 Thunnini), whose axial RM are internalised, located anteriorly and associated with *retia*.
273 Meanwhile, their ectothermic scombrid relatives such as mackerels (Scombrini) and bonitos
274 (Sardini) have laterally-located axial RM that lack *retia* [38,83,84]. Billfishes, including
275 marlins (Istiophoridae) and the swordfish (Xiphiidae), display brain heaters derived from the
276 superior rectus muscle [86,87], but show no trace of RM endothermy. Their endothermy is
277 therefore limited to the cranial region. The same configuration occurs in the butterfly mackerel
278 *Gasterochisma melampus* (Scombridae), which lacks RM endothermy but has a brain heater

279 derived from the lateral rectus muscle [7,38]. Tunas have elevated brain temperatures and
280 display *retia* in the cranial region: they have both cranial and axial RM endothermy [38,93].
281 However, the heat-generating mechanism for the cranial region of tunas is unclear: it might
282 pertain to the contraction of the red extraocular muscles [94], or to heat transfer from the axial
283 RM, via special openings in the back of the skull [38]. Only one tuna species, *Allothunnus*
284 *fallai*, has a true brain heater derived from all four rectus muscles [94]. Finally, the opahs
285 *Lampris* sp. (Lampridiformes) have been recently described to have a unique thermogenic
286 strategy involving both cranial endothermy [95] and a specific whole-body endothermy
287 hypothesized to be NST [96,97] (Supplementary Note 8).

288 While tunas and billfishes have long been classified together as “scombroids” [98],
289 molecular phylogenies support that they are not closely related within teleosts [99,100]. Within
290 Scombridae, the endothermic “true” tunas form a clade, but *Gasterochisma* is not their
291 immediate outgroup [101,102]. When adding opahs, this implies that endothermy appeared at
292 least four times in teleosts, and two or three times more in elasmobranchs (Fig. 1 – although
293 some analyses support that the common ancestor of lamnid and alopiid sharks was endothermic
294 [103]). Heater organs performing NST appeared three times independently: in billfishes,
295 *Gasterochisma*, and *Allothunnus*. Opahs likely developed another form of NST [97].

296 Endothermic elasmobranchs and teleosts share high levels of morphological and
297 ecological convergence. They tend to be pelagic, open ocean predators, powerful and fast
298 swimmers that travel over long distances and dive in colder water below the thermocline to
299 pursue their prey [38,104]. Endothermy may also be associated with larger body size [103,105].
300 Ecological niche expansion, towards broader temperature and geographical ranges is often
301 proposed as the main adaptive advantage of endothermy, and particularly RM endothermy, in
302 these taxa [38,81]. It might be the main driving force behind the convergent appearance of

303 endothermy in at least six different elasmobranch and teleost lineages, as well as in giant extinct
304 marine reptiles such as ichthyosaurs, plesiosaurs, and mosasaurs [33].

305

306 **5. Punctual thermogenesis in other vertebrates: heat production driven by lifestyle and**
307 **reproductive behaviour**

308 Shivering thermogenesis (ST) is a very common process in all mammals and birds, occurring
309 during long periods of low T_A or during the arousal phase of a torpor cycle (Supplementary
310 Note 4) to complement heat production achieved through NST [106]. It consists of heat
311 production through asynchronous excitation of muscle fibres, so that often muscles that oppose
312 one another are simultaneously active. This happens without external muscle work and allows
313 for all the ATP accumulated by myofilaments for contraction to be released as heat – a process
314 that also occurs for a fraction of ATP in normal contraction cycles (see a review of ST in [106]).
315 ST has evolved independently in birds and mammals (Table 1) and is considered a mechanism
316 of adjustment of T_B that was likely an evolutionary advantage in the acquisition of endothermy
317 for both groups [106]. However, it is also found in other clades able to achieve homeothermy
318 through specialized muscles. Among squamates, ST has been linked with parental care
319 behaviour in two species of snakes, the Burmese python (*Python bivittatus*) and the diamond
320 python (*Morelia spilota*; Supplementary Note 9). In both species, brooding females are able to
321 display muscle twitching behavior while tightly coiling around their eggs, resulting in ST that
322 can elevate T_B to a constant value of 30-32°C, i.e. warmer than T_A by about 7°C [107], and up
323 to 18°C [108]. This remarkable ability to thermoregulate has led some authors to suggest that
324 ST might not be the only mechanism involved in heat production in these two python species,
325 and that NST may also be possible [109]. Other python species have so far been experimentally
326 shown to be unable to achieve the ST homeothermy of Burmese and diamond pythons [110].
327 This suggests that this feature might be functionally linked with the acquisition of endothermy,

328 since the use of ST during arousal in small torpid mammals is very similar to that of
329 homeothermic brooding pythons [35].

330 The ability to temporarily maintain a higher T_B through NST has also been documented
331 in other squamates, i.e. in rattlesnakes during digestion, with a small elevation of T_B ($\approx 1^\circ\text{C}$ for
332 a large meal) directly correlated with meal size, that can last for up to 6 days [111], or in some
333 varanids ($\approx 0.5^\circ\text{C}$) [112]. In both examples, despite a strong increase in metabolic rate (3 to 4-
334 fold in varanids [112], 20-fold in some snakes [113]), the increased oxygen intake is dedicated
335 to physiological functions unrelated to heat production, such as elevating visceral blood supply.
336 Thus, increases in T_B always remain very small, also likely because of increased thermal
337 conductance [111], and cannot be used to monitor the rate of lowering T_B at the end of the
338 digestive process [111,112]. More recently, however, seasonal endothermy associated with
339 reproductive behaviour was reported in the black and white tegu (*Salvator merianae*), a large
340 (≈ 2 kg) burrowing lizard endemic to South America [114], providing additional support for an
341 ancestral rise of facultative endothermy linked with lifestyle constraints and parental care
342 (Supplementary Note 10) [11]. The independent acquisition of endothermy by several
343 vertebrate clades is thus likely to have been driven by a handful of key factors, including daily
344 heterothermy and ST, which themselves were subject to very similar environmental and
345 behavioural constraints [35,37].

346

347 **6. The leatherback sea turtle: homeothermy through large body size**

348 The leatherback sea turtle (*Dermochelys coriacea*), the largest extant turtle species (up to 700
349 kg [26]), has been described as homeothermic due to its ability to maintain its T_B at a relatively
350 constant level (at least 18°C higher than the ambient temperature of cold water [115]). This
351 thermoregulation is ensured by a very thick insulating layer of subepidermic vascularized
352 adipose tissue, analogous to the blubber in cetaceans and pinnipeds [116]. A blubber layer is

353 also present around the eyes near the retina, and inside the cranial cavity, protecting the
354 neurocranium and salt glands and limiting external heat loss [116]. Additionally, counter-
355 current heat exchangers, analogous to those of tunas and lamnid sharks, can be found in their
356 anterior and posterior flippers [115], and have been shown to provide active thermoregulation
357 through controlled heat dispersion, directly dependent on ambient water temperature (T_w)
358 [117]. These heat exchangers also function as a local heat retainer, maintaining the heat
359 generated through muscular thermogenesis inside the limbs and preventing hyperthermia in the
360 rest of the body [118]. These two adaptations allow the leatherback turtle to maintain its T_B
361 around 25°C, even during active swimming in cold water during deep dives, or in sub-polar
362 waters ($T_w < 5^\circ\text{C}$) [26,117].

363 The presence of endogenous heat production in the leatherback sea turtle is still debated,
364 however, as its physiological correlates of heat metabolism do not differ from those of other
365 reptiles (Supplementary Note 11). For this reason, large body size and high insulation have
366 been proposed as the main drivers behind the homeothermy of leatherback turtles, which would
367 allow heat conservation in the body without requiring a high metabolic rate [26]. This strategy,
368 labelled ‘gigantothermy’, has subsequently been proposed as a thermoregulatory process in
369 large non-avian dinosaurs [26,119–121]. Indeed, large body size has been described as a
370 potential driver of dinosaurian homeothermy since the late 1970s [74], and the gigantothermy
371 hypothesis has since been discussed in many palaeontological studies and reviews, with
372 contradictory interpretations on the possible influence of phylogeny and ontogeny on this
373 physiological process [15,112–114]. The highly specialized adaptations of the leatherback
374 turtle, however, are heavily linked with its constant swimming associated with an oceanic,
375 pelagic lifestyle. Other possible drivers for countercurrent exchange systems in the limbs of
376 tetrapods might also include a generally aquatic lifestyle, as documented in the platypus [46],
377 and extremely cold ambient temperatures, as observed in penguins [122] or in the Arctic fox

378 (*Vulpes lagopus*) [123]. The generalization of that strategy to other vertebrate clades on the sole
379 basis of their large body size is thus misleading, especially considering the size difference
380 between a leatherback turtle and large non-avian dinosaurs (e.g. sauropods).

381

382 **7. Conclusion: palaeophysiological inferences on the evolution of vertebrate endothermy**

383 As described above, vertebrate endothermy consists in multiple strategies that appeared
384 independently through time (Fig. 1). Many of these have only been scarcely investigated, are
385 not necessarily well-defined as endothermy in the literature, and are associated with a number
386 of constraints (parental care, size reduction, nocturnal bottleneck, niche expansion to colder
387 waters, etc.), currently still debated as potential drivers of endothermy among palaeontologists.
388 The various proxys used for palaeophysiological inference (Table 1) in this context have
389 provided many interesting results over the years, and in many cases several of these potential
390 drivers are likely to have jointly constrained the selection for higher BMR in several taxa [19].

391 In recent years, palaeophysiological inference has included a growing number of
392 phylogenetic comparative methods to include phylogenetic information in estimations and
393 reconstructions of metabolic rate [20,22,31,124]. Indeed, multiples “endothermies” have
394 evolved separately in the vertebrate lineage, each of them with its own adaptations to vastly
395 different lifestyles and different pathways to regulate heat production. Discussing the evolution
396 of endothermy in a large vertebrate clade (e.g. Archosauria, Synapsida) requires considering
397 the physiological differences between extant members of the crown group of interest, before
398 even trying to understand how this condition might have appeared in the last common ancestor
399 of this clade. As such, BMR is just another proxy to infer heat metabolism; a high BMR estimate
400 for a given fossil taxon does not provide *per se* evidence for endothermy in this taxon, nor a
401 precise picture of the physiological strategy found in the larger clade to which it belongs. For
402 this reason, we consider that inferences of endothermy in a specific fossil lineage should always

403 include a mention to a specific inferred mechanism, rather than simply defining several taxa as
404 endotherms or ectotherms based on similarity in BMR values, which is imprecise and might be
405 meaningless in a phylogenetic context. Discussions of hypotheses regarding such strategies in
406 such a context have allowed many recent physiological and palaeontological studies to unveil
407 a new, clearer picture of the evolution of vertebrate endothermy, of which this review is only a
408 short summary. Future methodological and conceptual developments will likely lead to
409 important changes in the understanding of such processes in the next few years [125].

410

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421

422 **Authors' Contributions**

423 Both authors conceived and designed this study, prepared figures and tables, authored and
424 reviewed drafts of the paper, and approved the final draft.

425

426 **Competing Interests**

427 We have no competing interests.

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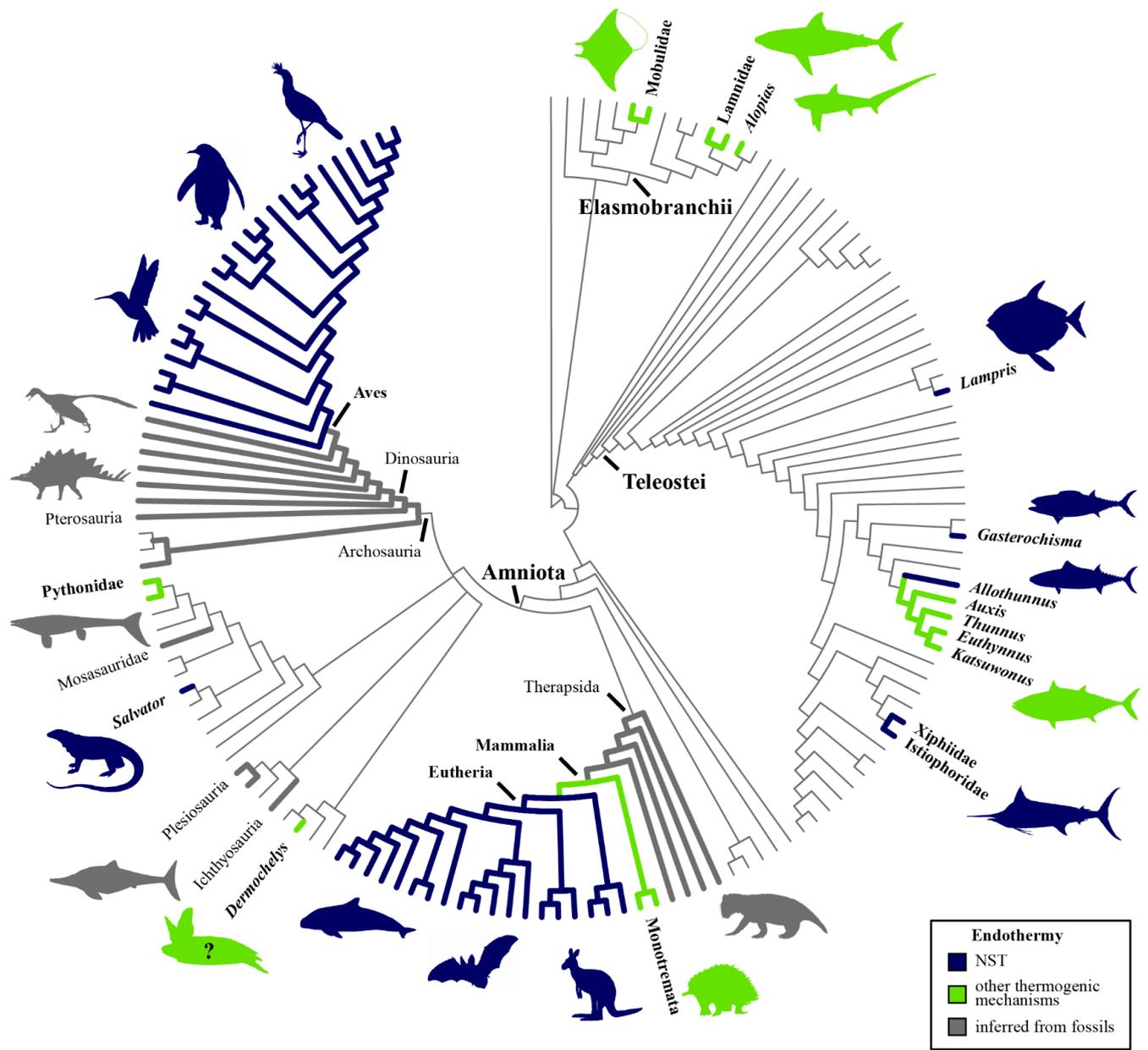
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794 **Figure captions**

795 FIGURE 1. Phylogeny of vertebrates showing independent appearances of heat production
796 strategies described as endothermy in the literature, with taxa where endothermy has appeared
797 at least once being labelled in bold. A distinction is made between non-shivering thermogenesis
798 (NST), i.e. the strategy most commonly referred to as endothermy (see text), and other
799 strategies; some taxa, such as mammals and birds, are able to achieve NST, but can also
800 occasionally rely on other mechanisms to generate body heat (see text). Fossil taxa that have
801 been inferred as potential endotherms are also labelled. The leatherback sea turtle is labelled
802 with a question mark, since NST has not been convincingly demonstrated in that species.
803 Reference phylogeny compiled from several sources [102,126–131]; silhouettes of terminal
804 taxa obtained from PhyloPic (<http://phylopic.org/>).

805



807

808 TABLE 1. Summary of physiological strategies described as endothermy in the literature for

809 each vertebrate taxon discussed in the text, with emphasis on phylogeny, cellular/molecular

810 mechanisms (if known), and palaeophysiological proxys for associated fossil stem-groups.

Taxonomy	Endothermic lineage	Type of endothermy	Anatomical specialisation	Thermogenic mechanism	Possible palaeophysiological proxys
Elasmobranchii, Lamniformes	Lamniidae (mackerel sharks) [89]	Red muscle (RM) endothermy, cranial endothermy [89]	Internalised axial RM, axial <i>retia</i> , vascular connection to braincase [90]	Byproduct of RM contraction [89,90]	None known, maybe body size and palaeobiogeography [105]
Elasmobranchii, Lamniformes	<i>Alopias vulpinus</i> (common thresher shark) [91]	RM endothermy [91]	Internalised axial RM, axial <i>retia</i> [91,104]	Byproduct of RM contraction [91,104]	None known
Elasmobranchii, Myliobatiformes	Mobulidae (manta rays) [92]	Cranial endothermy (suggested) [92]	Cranial <i>retia</i> [92]	Unknown	None known
Teleostei, Lampridiformes	<i>Lampris</i> sp. (opahs) [96]	Whole-body endothermy [96], cranial endothermy [95]	Specialised pectoral RM, insulating adipose tissue, gill arches <i>retia</i> [96]	Byproduct of RM contraction [96], proposed NST in the sarcoplasmic reticulum (sarcolipin) [97]	Osteocytic bone [132,133]
Teleostei, Istiophoriformes	Istiophoridae, Xiphiidae [86]	Cranial endothermy [86]	Heater organ (modified ocular muscles), cranial <i>retia</i> [86,87,101]	NST in the sarcoplasmic reticulum (ryanodine receptor) [88]	High remodelling in bone of the rostrum [134]
Teleostei, Scombriformes, Scombridae	<i>Gasterochisma melampus</i> (butterfly mackerel) [7]	Cranial endothermy [7]	Heater organ (modified ocular muscles), cranial <i>retia</i> [7]	NST in the sarcoplasmic reticulum [88]	None known
Teleostei, Scombriformes, Scombridae	Thunnini (“true” tunas) [84]	RM endothermy, cranial endothermy [83,84,93]	Internalised axial RM, axial and cranial <i>retia</i> [38,83,84], heater organ (only in <i>Allothunnus fallai</i>) [94]	Byproduct of RM contraction [38], NST in the sarcoplasmic reticulum (<i>A. fallai</i>) [94]	Osteocytic bone [133]
Amniota, Mammalia	Monotremata (monotremes) [40]	Facultative endothermy, shivering [35,40]	Insulating fur and fat, four-chambered heart, countercurrent vessels in limbs (platypus) [40,46]	Shivering [106]	Fur, respiratory turbinals [135], specific bone histological features [136]
Amniota, Mammalia	Metatheria (marsupials) [137]	Whole-body endothermy, shivering [41,137]	Insulating fur, respiratory turbinals, four-chambered heart [2,11]	Shivering, NST (mechanism unknown) [50,138]	Fur, respiratory turbinals [135], specific bone histological features [139]
Amniota, Mammalia	Eutheria (placental mammals) [2]	Whole-body endothermy, shivering [2]	BAT, insulating fur or blubber, respiratory turbinals, four-chambered heart [2,11]	Shivering, NST in BAT (UCP1)[43,106] – albeit with	Fur, respiratory turbinals [135], specific bone histological features [139]

				exceptions [42,52], NST in skeletal muscles (sarcolipin) [140]	
Amniota, Squamata, Pythonidae	<i>Python bivittatus</i> (Burmese python), <i>Morelia spilota</i> (diamond python) [109]	Muscle thermogenesis [109]	Specialised muscles [108]	Byproduct of muscle contraction, possible NST [109]	None known
Amniota, Squamata, Teiidae	<i>Salvator merianae</i> (black and white tegu) [114]	Seasonal reproductive endothermy [114]	Unknown	Unknown (proposed NST) [114]	None known
Amniota, Chelonia	<i>Dermochelys coriacea</i> (leatherback sea turtle) [26]	Gigantothermy [26]	Insulating adipose tissue, counter-current vessels in flippers [116,141]	Probably byproduct of muscle and metabolic activity [141]	None known (traces of blubber detected in extinct marine amniotes) [142]
Amniota, Archosauria	Aves (birds) [10]	Whole-body endothermy, shivering [4,10]	Insulating feathers, respiratory turbinates, unidirectional airflow in lungs, muscle hyperplasia, four-chambered heart, small genome size (debated) [2,57,58,68]	Shivering, NST in skeletal muscles (ANT, sarcolipin) [57,60,106]	Feathers, respiratory turbinates, specific bone histological features, bone pneumaticity (respiratory air sacs), [28,29,68,72,77,143]

Supplementary information

for

‘The evolution of mechanisms involved in vertebrate endothermy’

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Supplementary notes

Note 1 – Mitochondrial heat production in placental mammals

Brown adipose tissue (BAT), a structure only found in placental mammals, is much more vascularized than the more commonly distributed white adipose tissue, and brown adipocytes present a higher number of lipid droplets than white ones [1,2]. Additionally, brown adipocytes present a high number of mitochondria, where heat is generated through the activity of UCP1 (Uncoupling Protein 1), also known as thermogenin [1]. UCP1 is a mitochondrial transmembrane protein, activated by free fatty acids [3] released from triglyceride droplets, which in turn is activated by the binding of norepinephrine on β -adrenergic receptors in brown adipocytes [2,4]. Production of norepinephrine is induced by cold-sensitive thermoreceptors via the hypothalamus and the sympathetic nervous system [4]. In mitochondria, the proton flux generated by the respiratory chain is released in the intermembrane space. This creates a difference in both charge and concentration between the inside and outside of the inner mitochondrial membrane, which results in an electrochemical gradient known as the proton motive force [4]. In most cells, this proton motive force is used by ATP-synthases to produce adenosine triphosphate (ATP) through of the proton flux inside the mitochondria; ATP is then exported in the cytosol. In BAT, UCP1 recaptures part of the proton flow, thus uncoupling the proton motive force and dissipating it as heat [2,4,5].

Note 2 – Inferred cases of endothermy in non-shivering thermogenesis (NST) in marsupials

Norepinephrine-activated adaptive NST has been identified in several marsupial species, including the Tasmanian devil (*Sarcophilus harrisii*) and the eastern barred bandicoot (*Perameles gunnii*), showing the existence of an alternative metabolic way to achieve adaptive NST in mammals [6,7]. The authors of these studies hypothesized that uncoupling proteins 2 (UCP2) and 3 (UCP3), closely related to UCP1 and also involved in mitochondrial respiration uncoupling, might be involved in marsupial NST, since their expression has been found to be linked with increasing NST in another marsupial species, the Tasmanian bettong (*Bettongia gaimardi*) [7,8]. The potential roles of UCP2 and UCP3 in heat production, however, have never been shown, as the functions of both proteins have been poorly studied [4,9]. Alternatively, some studies have suggested that monotremes and marsupials might achieve homeothermic endothermy primarily through high insulation and skeletal-muscle-based thermogenesis (see a detailed description of muscular thermogenesis in section 3 of the main paper and Supplementary Note 5) [10,11], both of which are commonly distributed among vertebrates [11,12].

Note 3 – Independent losses of UCP1 in placentals

The inactivation of the *ucp1* gene has occurred independently in several clades of placental mammals, i.e. cetaceans, xenarthrans, pigs, horses, pangolins, elephants, sirenians, and hyraxes [13,14]. These losses of thermogenin activity may have been induced by the need for precociality, since all of those lineages but one (pangolins) are precocial [13]. The inactivation of *ucp1* has also resulted in low diversification rates and large body sizes in aforementioned clades compared to other placentals, but the presence of functioning UCP1 in many other clades of large mammals suggests a number of other evolutionary constraints are likely involved [13]. All clades involved present highly specialized diets (e.g. feeding on small arthropods associated with complete loss of teeth in pangolins, anteaters, and baleen whales) and/or lifestyles (e.g. aquatic for cetaceans and sirenians, fossorial for some xenarthrans and pangolins) that might also have been important constraints on the evolution of *ucp1* [13]. Nevertheless, all those groups maintain a strong ability to thermoregulate, albeit with some variations (e.g. many xenarthrans heavily rely on ST in response to cold and have a relatively low body temperature [14]; hyraxes can lower their basal metabolic rate (BMR) and body temperature (T_B) depending on variations in ambient temperature (T_A), and display basking behavior as an energy conserving mechanism [15,16]). Skeletal muscle thermogenesis has also been suggested as an alternative strategy for those clades [13], with activation mechanisms likely to be sarcolipin-dependent, as already described in some mammals and birds (see section 3 of the main paper and Supplementary Note 5).

Note 4 – Daily torpor and hibernation in mammals and birds

In mammals, daily torpor has been documented in small species, such as marsupial dunnarts [17], rodents [18], bats [19], or small lemurs [20], during which metabolic rate is reduced to 20-30% of its normothermic value, for a period that can last up to 17 hours [20]. Daily torpor has been shown to be directly initiated by low values of T_A , with the duration of the torpor state being shortened when T_A subsequently increases, and T_B and MR values are thus only passively regulated [21–23]. Daily torpor has also been shown to be linked with circadian and circannual rhythms through photic stimuli, but these factors appear to play a larger role in arousal from torpor than in torpor entry, which is also strongly regulated by environmental constraints, such as latitude or food and water availability [21,24].

In birds, daily torpor (predominantly during nighttime) has been described in many species, with a strong focus on torpor in hummingbirds (family Trochilidae), a large clade with a set of unique ecological adaptations constrained by extreme lifestyle compromises [25–28]. Many hummingbird species weigh less than 5 g [27], and present an extremely high metabolism constrained by a high consumption of sugar (flower nectar contains up to 38% sugar [27]); adults feed more than a hundred times a day ($\approx 20\%$ of daylight hours [27]), and hovering behaviour exhibited during feeding phases is energetically very costly (MR = 4 mL O_2 /g/h, which can increase 10-fold during hovering and forward flight – the highest mass-specific metabolic rate among vertebrates [29]). Muscular activity is also extremely high during hovering phases: heart rates can increase to 1300 beats per min, and wing beat frequencies to 80 per second [29], both of which are ensured through hypertrophy of pectoralis and supracoracoideus muscles, constituting about 30% of body mass together [29]. For this reason, it has been suggested that hummingbirds produce their metabolic heat mostly through SR-based muscular NST [25]. During steady-state foraging, several species of hummingbirds have been shown to make use of recently-ingested sugar through oxidation of both glucose and fructose, switching from endogenous free fatty acids to exogenous sugars (up to 100% of the energy used to fuel their hovering behaviour [30]), and completing the turnover of ingested sugar in about 30 min, i.e. more rapidly than any other vertebrate [31].

Since maintaining such a high metabolism 24 hours a day would be impossible, especially when T_A is low, hummingbirds go into a state of torpor when they are inactive during the night.

The lowering of BMR is achieved through passive lowering of T_B from more than 40°C to 12–20°C, as in torpid mammals. However, it is actively maintained through increased oxygen transport rates to compensate for the slower heart beat induced by lower T_B , which is facilitated by the increased heart size of hummingbirds compared to that of other birds [28]. During the arousal phase, metabolic rates increase to a level comparable to that achieved during hovering flight [28], which is achieved through active muscular NST, and before reaching non-torpid values of T_B [25]. This high metabolism cannot be sustained during night time, during which hummingbirds enter a state of torpor, occurring in two phases – an initial drop of oxygen consumption hypothesized to be associated with stopping cold-induced ST, followed by a slower diminution in metabolic rate. This decrease in BMR has been shown to occur passively, but is actively maintained through increased oxygen transport rates [28].

Daily torpor in hummingbirds is thus a highly specialized and actively-controlled process, very different from what can be observed in daily heterothermic mammals. While it is well established that torpor might be a plesiomorphic feature among amniotes, facilitating the acquisition of “partial” endothermy in early synapsids [10,32], studies on this matter have focused mostly on mammalian endothermy, and the highly autapomorphic features of hummingbird torpor might not necessarily be associated with the daily heterothermy supposedly acquired at a more inclusive level of the tree [21]. Hence it is possible that daily heterothermy did appear independently in birds and mammals [22], and the general definition of daily heterothermy should be put in the more specific context of subsequent adaptations of torpid metabolism associated with the ecological constraints of each species when discussing its evolution in mammals and birds [21].

Hibernation, i.e. torpor lasting for several days to several weeks, during which the animal does not forage and relies on food caches or internal energy reserves to survive [21], is a completely different process. Contrary to daily torpor, mammalian hibernation is found in species with a much larger range in body mass, causes metabolic rate to drop down to only 6% of its normothermic value [21], and involves an active physiological control to maintain BMR and T_B at these low values [4,21]. Factors hypothesized to play a part in this active control include e.g. lowering of pH to reduce BAT mitochondrial uncoupling, and cessation of carbohydrate oxidation due to reduced activity of pyruvate dehydrogenase [33]. Hibernators also exhibit increased capacities of cardiac storage and release of Ca^{2+} , as well as periods of prolonged apnea, neither of which are well understood [33]. During the arousal phase to rewarm the animal and leave the torpid state, hibernating mammals will activate heat production through NST in BAT, which will achieve a local temperature higher than T_B by up to 14°C [2], until the animal is entirely rewarmed, sometimes with additional ST [2]. Conversely, arousal from daily torpor has been shown to be strongly regulated by the circadian control of T_B (which is suppressed during hibernation in hibernators), and does not involve any additional metabolic activity linked with BAT [21]. This does not prevent, however, some species to alternate between daily torpor and hibernation depending on environmental conditions [34].

In birds, despite hypothesized convergences with mammals regarding nocturnality and intermediate metabolism, birds do not present extended periods of torpor analogous to hibernation in mammals; only one species, the common poorwill (*Phalaenoptilus nuttalli*), a nightjar (Caprimulgidae), has been described as lowering their T_B for long periods of time (≥ 10 days) during winter [35]. The periods of torpor in the common poorwill can last 10 to 25 days during winter, and are interrupted by short periods of activity about every five days [35], similarly to what can be observed in many hibernating mammals [21]. Arousing from torpor may also be occasionally caused by passive solar warming [35], which is not usually considered a marker of mammalian hibernation [2]. However, all hibernating mammals, unlike common poorwills, hibernate in sheltered locations and thus cannot be aroused from torpor from sun exposition. Furthermore, common poorwills remain inactive during such arousal periods and

do not seem to depend on it to regulate their torpor dynamics [35], meaning they can be described as hibernating birds. It has been hypothesized that hibernation in the common poorwill might be linked with its hot and arid habitat [35], but several species of closely-related nightjars with a similar ecological lifestyle do not exhibit hibernating behavior, despite being able to achieve daily torpor, and other factors may thus be implied in this only avian occurrence of hibernation [36].

Note 5 – Mitochondrial heat production in birds

The activation of ANT-induced muscular thermogenesis in birds during embryogenesis involves the peroxisome proliferator-activated receptor- γ coactivator-1 α (PGC-1 α), a protein that is also a key metabolic regulator in mammals [37]. The release of PGC-1 α is controlled by thyroid hormones in mammals, and regulates mitochondrial biogenesis and cellular respiration rates, among many other functions. In birds, it has been shown to be similarly regulated by thyroid hormones, but these hormones do not appear to have a direct role in the regulation of uncoupling activity in ANT [37]. Despite some similarities in the mechanisms involved in cellular heat production, mammals and birds thus display completely different strategies at a molecular level.

Note 6 – Sarcolipin-activated heat production in red muscles

The ability to passively generate heat as a byproduct of striated muscle activity is a well-known process that has been described in many vertebrates [11,38], and several of its specializations to locally increase heat production and ensure whole-body homeothermy through subsequent heat redistribution have been studied extensively, most notably in several actinopterygians and chondrichthyans [38–41] (see also section 5 of the main paper). This process involves the uncoupling of calcium ions (Ca^{2+}) recapture in the sarcoplasmic reticulum (SR), a specialized calcium-storing structure in muscle cells, involved in the activation of skeletal muscle contraction. In a normal muscular contraction cycle, SR releases Ca^{2+} in the cytoplasm; these ions then bind to troponin C, which exposes the binding site of myosin on actin filaments [42]. After binding to an actin molecule, each myosin binds an ATP, which causes its head to detach from the actin and start a new cycle until muscle contraction is complete [11,38]. The Ca^{2+} used in the process is then recaptured in SR through a specialized protein, the sarco/endoplasmic reticulum Ca^{2+} -ATPase (SERCA), which also consumes ATP in the process. The uncoupling of this recapture involves blocking the Ca^{2+} recapture by SERCA without preventing its dephosphorylation of ATP; the energy produced by this dephosphorylation is thus not used for Ca^{2+} recapture, but dissipated as heat by the cell [11,38,43].

The molecular mechanism involved in blocking the calcium flux in SERCA has been the subject of many hypotheses over the last decades [38]; recently, several studies have shown that sarcolipin, a protein that binds to SERCA to prevent calcium recapture, is responsible for SR uncoupling in muscular thermogenesis, and thus a key regulator of muscular NST in vertebrates [11,43,44]. This process has been shown to complement mammalian NST in BAT in mice when exposed to cold, resulting in two simultaneous processes to achieve heat production in both adipose tissue and skeletal muscles [45,46], and has been hypothesized as a way to achieve facultative homeothermy in early mammals before they evolved BAT-based NST [11]. The role of sarcolipin in adaptive thermogenesis, however, has yet to be confirmed by *in vivo* experiments, and its importance in NST for mammals, birds, and vertebrates in general remains elusive [47].

Note 7 – Metabolism of flightless birds

The acquisition of flightlessness in birds involves a series of highly convergent adaptations, such as a high amount of subcutaneous fat, and a relatively large body size compared to that of

closely-related taxa [48] – two features also involved in the heat metabolism of aquatic mammals, and of the leatherback turtle, another amniote species able to achieve homeothermy independently of muscular NST (see sections 5 and 6 of the main paper). The loss of flight in kiwis in particular has been linked with the need for energy conservation in the harsh environmental conditions of New Zealand in the Late Cretaceous, as well as the lack of mammal predators [48], resulting in a nocturnal lifestyle that may have conditioned their intermediate metabolic rate, analogous to the optional homeothermy linked with facultative nocturnality described in echidnas [10] and inferred in the first endothermic mammals [32]. The kakapo (*Strigops habroptilus*), a flightless nocturnal parrot also endemic to New Zealand, has been inferred to present a similar strategy, but further research would be needed to confirm this hypothesis [49,50].

Note 8 – Physiology of the opahs (*Lampris* sp.)

Unlike tunas and lamnid sharks, opahs do not use their axial musculature to produce heat. Instead, the main centre of thermogenesis is the red pectoral fin muscles, constantly contracted during swimming. Heat is insulated by a thick layer of subcutaneous fat, and kept within the body by *retia* located in the gill arches that redistribute it in the whole circulatory system [51]. Contrary to the other endothermic teleosts, opahs then warm up their entire body rather than more localised regions. Another peculiarity of the opah endothermy is that their pectoral musculature seems to be divided in two categories: light red contractile muscles involved in pectoral fin flapping, and dark red muscles that display very high levels of sarcolipin and might be able to produce heat without contracting, via futile calcium cycling [52]. Muscular endothermy in the opahs would then be remarkably similar to that of birds, for example (see section 2), and a form of NST.

Note 9 – Facultative endothermy in brooding pythons

In Burmese and diamond pythons, shivering cycles last for about 12 h/day for $T_A = 28^\circ\text{C}$, and around 8 h/day for lower T_A [53]. The number of muscular contractions during shivering has been shown to be correlated with the increase in oxygen consumption at low T_A , which can reach a peak of 0.088 mL O_2 /g/h for female Burmese pythons, i.e. a 22-fold increase compared to a non-brooding female in similar environmental conditions [54]. Maintaining a high T_B is also achieved through on periods of basking on clear days, but T_B does not change significantly between clear and cloudy days, suggesting homeothermic abilities analogous to those of mammals and birds [55]. Similarly, variations in nest temperature linked with variations in T_A do not seem to have an impact on T_B during shivering phases, as clutch temperature decreases with decreasing T_A and does not seem to influence T_B regulation [56].

Note 10 – Seasonal endothermy in tegu lizards

Black and white tegus (*Salvator merianae*) are able to maintain their T_B at a constant value of about 30°C during the 3 months of their reproductive cycle, with $T_B - T_A \approx 6.5^\circ\text{C}$. Neither variations in environment type (i.e. being inside or outside their burrows), nor activity patterns or feeding status have any influence on T_B during that period, showing that body heat is endogenously produced through NST [57]. While metabolic rates and thermal conductance has yet to be measured in this species, all known parameters related to heat metabolism strongly support the presence of true endothermy in tegu lizards, with a heat production strategy likely to be similar to at least one of those present in mammals and birds. Interestingly, as a large fossorial species with facultative endothermy linked with seasonal reproduction, the black and white tegu presents a close similarity in lifestyle with the echidna, which further supports the hypothesis of facultative endothermy having appeared as an exaptation linked with lifestyle constraints in otherwise ectothermic clades, before the acquisition of true endothermy in crown

mammals and birds [10,57,58]. Since female tegus remain with their clutches through the incubation period, the parental care hypothesis is also a good explanatory factor of the acquisition of NST in that species [57]. Further research, however, is needed to determine the structures associated with NST in tegus, as well as the hormonal activation and subsequent intracellular mechanisms involved in their heat production strategy.

Note 11 – Physiology of the leatherback sea turtle

The leatherback sea turtle's abilities to thermoregulate have been hypothesized to be linked with regional endothermy in its limb and pectoral muscles, which maintain a higher thermal gradient than the rest of the body, likely because of constant swimming activity [59]. A similar ability has also been described in another marine turtle species, the green sea turtle (*Chelonia mydas*), but this species is not able to maintain high muscular activity associated with homeothermy for $T_w < 20^\circ\text{C}$ [59]. The explaining factor behind the unique homeothermic abilities of the leatherback turtle compared to that of its sister-groups has thus been the subject of many hypotheses, but so far no heat production strategy associated with it has been clearly identified. The adipose tissue layer in leatherback turtles has been described by some studies as BAT, analogous to that of placental mammals, and hypothesized to be capable of NST [60], but no evidence of such a mechanism has been found so far. Furthermore, a recent study found no significant difference in the allometry of body mass and mass-specific resting metabolic rate (BMR) between leatherback turtles and other marine turtle species, nor between 'reptiles' in general, but a significant difference was found between 'reptiles' and mammals [61]. Two studies [61,62] found low mass-specific BMR values for leatherback turtles compared to that of mammals, and values of maximal metabolic rates (MMR) equivalent to those of mammalian BMR. Furthermore, the muscle citrate synthase activity (another commonly used proxy for heat metabolism) of leatherback turtles also falls within the normal range for other reptiles [63]. Interestingly, most non-amniote vertebrates are also aquatic and ectothermic, the only exception being large predators with a highly active lifestyle. While endothermy in teleosts and chondrichthyans has been extensively studied (see section 4 of the main paper), the lack of endothermic non-amniote tetrapods has so far received limited attention. The high correlation between resting and maximum rates of oxygen consumption in anurans suggests that aerobic capacity would be the main driver behind a potential acquisition of endothermy in early tetrapods [64]. The sedentary lifestyle of most amphibians and related taxa might thus have prevented such an acquisition [42], but further research is needed to investigate this hypothesis.

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