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Cancer prevalence is remarkably low in turtles and is correlated with life history traits in birds, mammals, and squamates

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ABSTRACT

Cancer rates vary widely across vertebrate groups. Identifying species with lower-than-expected cancer prevalence can help establish new models for unraveling the biological mechanisms underlying cancer resistance. Theoretical predictions suggest that cancer prevalence should be positively associated with body mass and longevity in animals. Yet, in mammals, the best studied vertebrates in terms of cancer, this prediction does not hold true: a phenomenon known as Peto's paradox. Despite mounting work disentangling the biological basis of Peto's paradox, it is still relatively unknown whether other major vertebrate groups behave similarly to mammals or might hold new keys to understanding cancer biology. Here, we present the largest dataset available so far on cancer prevalence across all major groups of tetrapod vertebrates: amphibians, birds, crocodilians, mammals, squamates (lizards and snakes), and turtles. We investigated cancer prevalence within and among these groups and its relationship with body mass and lifespan. This is the first study to analyze non-avian reptile groups separately. We found remarkably low cancer prevalence in birds, crocodilians, and turtles. Counter to previous studies, we found that body mass and lifespan are inversely related to cancer prevalence in mammals, although Peto's paradox still holds true in this group. Conversely, we rejected Peto's paradox in birds and squamates, as neoplasia prevalence was positively associated with body mass in these groups. The exceptionally low cancer prevalence in turtles and extensive variation in cancer prevalence amongst vertebrate families hold particular promise for identifying species with novel mechanisms of cancer resistance.

KEYWORDS:

Comparative oncology, life history, neoplasia, Peto's paradox, tetrapods, zoo

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INTRODUCTION

Cancer is thought to occur in virtually all vertebrates, but not all vertebrates are at equal risk of developing cancer (Aktipis et al., 2015; Leroi et al., 2003). Based on theoretical predictions of multicellularity (Peto et al., 1975), larger animals with many cells should be at greater risk of developing cancer than animals with fewer cells. Similarly, organisms with long lifespans have more time to accumulate cancer-causing mutations than organisms with shorter lifespans (López-Otín et al., 2013; Peto et al., 1975; Tollis et al., 2017). Consistent with these expectations, cancer prevalence increases with both body size and age within certain species such as humans and dogs (Vazquez & Lynch, 2021). However, at the interspecific level, other studies have found no correlation between body size, lifespan, and cancer prevalence in mammals (Boddy et al., 2020a; Vincze et al., 2022) and possibly other major vertebrate groups (Harris, 2022): a phenomenon known as Peto's paradox (Nunney et al., 2015; Peto et al., 1975). To explain this seeming contradiction, animals at these upper life history extremes must have developed molecular mechanisms that offset their increased cancer risk (Caulin & Maley, 2011). Therefore, examining variation in cancer prevalence within and among vertebrate groups can help identify species that have evolved promising anti-cancer mechanisms (Chiari et al., 2018).

Multiple studies have reported variation in cancer prevalence across vertebrates, with mammals having the highest prevalence followed by reptiles, birds, and amphibians, respectively (Effron et al., 1977; Madsen et al., 2017; Møller et al., 2017; Pesavento et al., 2018). There is also evidence of variation in prevalence within major vertebrate groups (Boddy et al., 2020b). For instance, carnivores have higher cancer prevalence than other placental mammals (Vincze et al., 2022), while opossums have remarkably high cancer prevalence compared to any other mammal (Boddy et al., 2020a). Among non-avian reptiles, turtles appear to have lower prevalence than snakes and lizards (Garner et al., 2004; Sykes & Trupkiewicz, 2006). Data for crocodilians is sparse, but it appears that they may also have very low cancer

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prevalence (Boddy et al., 2020b; Garner et al., 2004). However, most large-scale comparative studies focus on estimating differences in cancer prevalence among, rather than within, major vertebrate groups (but see (Harris, 2022)). Moreover, no previous studies have investigated the major groups of non-avian reptiles individually (crocodilians, squamates, and turtles). Beyond studying species that are particularly large and/or long-lived, a fuller accounting of cancer prevalence within major vertebrate taxa makes it easier to study physiological, ecological, and cellular mechanisms that underlie variation in cancer prevalence across animals.

Here we present the first study comparing cancer prevalence within and between tetrapods, which comprises all the extant major vertebrate groups except fish. We obtain necropsy data, including cancer incidence, from mammals, birds, amphibians, turtles, crocodilians, and squamates (lizards and snakes) from multiple zoos as well as from previous publications (Boddy et al., 2020a; Duke et al., 2022). Using the largest dataset to date for most tetrapod groups, we then analyze cancer prevalence within each group in relation to phylogeny, variation in body mass and lifespan among species, and intrinsic cancer risk estimated from species life history traits. Since prevalence is a proportion, we use phylogenetic comparative methods that are specific to this kind of response data, which is expected to follow a binomial distribution (Paradis & Claude, 2002), whereas previous studies have typically treated prevalence as continuous data (e.g., Boddy et al., 2020a; Vincze et al., 2022).

We found no cases of cancer and one case of neoplasia in both turtles and crocodilians, and very low cancer prevalence in birds. Within all groups except turtles and crocodilians, we identified some families with remarkably high neoplasia and cancer prevalence. Finally, we found that Peto's paradox does not apply to birds and squamates, and that body mass and lifespan inversely influence neoplasia and cancer prevalence in mammals. These are new and remarkable findings showing how cancer prevalence varies tremendously within and among vertebrate groups and is influenced by life history traits.

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MATERIALS AND METHODS

Cancer data collection and curation

Following Aktipis et al. (2015), we defined any neoplastic growth as a cancer-like phenomenon. We searched paper reports or digital databases of three European zoos (Allwetter, Münster, Germany; ZOOM Erlebniswelt Gelsenkirchen, Gelsenkirche, Germany; Rotterdam Zoo, Rotterdam, The Netherlands) and one in the United States (Birmingham Zoo, Alabama) for necropsies recorded between 1998 and 2019 (**Appendix Table 1**). The databases for Allwetter, ZOOM Erlebniswelt, and Birmingham Zoo were all local databases while Rotterdam Zoo data was obtained through local paper reports and the Species360 Zoological Information Management System (ZIMS) with Rotterdam Zoo's authorization. We also incorporated the mammalian necropsy reports from Boddy et al. (2020a) and a dataset of neoplasia (benign or malignant) in snakes from Duke et al. (2022). The dataset of Duke et al (2022) is based on cancer prevalence calculated from the number of biopsies and necropsies on the total number of individuals (live and dead) of each species housed at a given time at participating institutions in the study. Since this prevalence was partly based on live individuals and not strictly on necropsy reports, as was the rest of our data, we performed subsequent analyses both with and without the data from Duke et al. (2022).

The list of keywords used for the search of neoplasia within the necropsy reports can be found in the file "Neoplasia_Terms" (*Dryad after manuscript acceptance*). To build the dataset for this study, we only retained data for tumors if these were confirmed by histological reports done by veterinary pathologists. Any neoplastic individual that was too autolytic to diagnose potential cancer incidence was removed from the study altogether. Any juveniles that died during the first month of life were removed from the dataset since they would have a low risk of

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developing cancer and would bias the data toward lower cancer prevalence. We tallied the total number of necropsies, independently of whether they had a tumor or not, for each species represented by a necropsy report during the time frame considered in this study (1998-2019). The data were then organized into cases of "neoplasia", which is defined as any instance of neoplasia, benign or malignant, as diagnosed through veterinary pathology and confirmed by histology, and "cancer", which is defined as any malignant neoplasia, typically diagnosed by the presence of pathologies such as abnormal cellular nuclei, growth into surrounding tissues, and/or metastasis of tumor cells. The data were further curated to include only species that could be matched with a published phylogeny for their group (see below) and those species for which at least one life history trait (lifespan and/or body mass) could be found. Finally, two species, *Rousettus aegyptiacus* (mammal) and *Thamnophis radix* (squamate from Duke et al. (2022)) had more than 500 necropsies and were removed from the dataset as they prevent convergence in downstream comparative phylogenetic statistical analysis (see below).

"Neoplasia prevalence" per group was calculated as the total number of neoplasias divided by the total number of necropsies for each group following the rate of tumorigenesis calculation in Wagner et al. (2020). "Cancer prevalence" per group was calculated as the total number of cancers divided by the total number of necropsies for each group. "Malignancy prevalence" per group was calculated as the total number of cancers divided by the total number of neoplasias for each group also following (Wagner et al., 2020). Finally, although malignancy requires neoplasia to occur first (Wagner et al., 2020), our data are based only on deceased individuals (i.e., necropsies). Thus, the number of neoplasias could be skewed if the individual did not die from it or other causes. For that reason we report neoplasia, cancer, and malignancy prevalence values throughout our results.The full dataset can be found on Dryad (*after manuscript acceptance*).

Life history data collection

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Maximum body mass and lifespan were collected for each species when available. Each species included in the dataset had at least one of these traits available. The Animal Aging and Longevity Database, AnAge (Tacutu et al., 2018), was searched for species present in our necropsy reports. If no species data was found in AnAge, and instead a primary source was found, then the primary source was used. Data origin for the life history trait information is listed in the full dataset file under "Sources LHT" for each group (*Dryad after manuscript acceptance*). If no source for a verified maximum lifespan or body mass could be found, then that species was removed from analyses. Most of the amphibian life history traits were sourced from the AmphiBIO database (Oliveira et al., 2017). As body mass information was lacking for most amphibians, snout-vent length was used as a proxy for body mass as it was the most common measurement for amphibian body size.

Intrinsic Cancer Risk Analysis

Intrinsic cancer risk (ICR) due to body size and lifespan was estimated for each species in the dataset following Peto's (2015) model, where ICR = lifespan^6×body mass. Only species for which both body mass and lifespan data are both available could be included in this analysis. ICR values were log scaled and transformed using a min-max normalization (ICR – min(ICR))/(max(ICR) – min(ICR)) to bring them to a 0-1 range within each group, where 0 represents the species with the lowest intrinsic cancer risk and 1 represents the species with the highest intrinsic cancer risk within each lineage. To test the relationship between expected cancer risk and observed neoplasia, cancer, and malignancy prevalence, we used generalized estimating equations (*compar.gee*, R package {ape}) (Paradis et al., 2004; Paradis & Claude, 2002) between the log scaled intrinsic cancer risk and neoplasia, cancer, or malignancy prevalence for each group using R v4.1.1 (R Core Team, 2018).

Statistical Analyses

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The Shannon-Wiener Diversity Index and the Shannon Equitability Index were used to assess species diversity and the evenness of species distribution in our dataset (Shannon, 1948; Tuomisto, 2012). The Shannon-Wiener Diversity Index represents the proportion of species that make up the population of each group and thus the average amount of diversity for the group. The Shannon-Wiener Diversity Index (H) is calculated as follows:

$$H' = -\sum_{i=1}^{S} p_i \ln p_i$$

Where p_i is the relative abundance of species i, S is the total number of species present in the group and ln is the natural log. The evenness was then calculated using the Shannon Equitability Index: $E_H = H/\ln(S)$. The Shannon Equitability Index is a measure of how even individuals (i.e., necropsies) are distributed amongst the species of each group. A value of 1 means all species are equally represented by the same number of necropsies, while a value closer to 0 means that one or a small number of species are overrepresented.

To estimate whether prevalence of neoplasia, cancer, and malignancy are significantly different among groups, we calculated the 95% confidence intervals for each group prevalence value for neoplasia, cancer, and malignancy separately. Prevalence of neoplasia, cancer and malignancy among tetrapod groups were compared using the test of equal proportion using the function *prop.test*.

To assess the influence of lifespan and body mass on neoplasia, cancer, and malignancy, statistical analyses were run for the neoplasia prevalence, cancer prevalence, and malignancy prevalence. Analyses were run for the entire dataset and then again only considering species with ≥5 or ≥10 necropsies per species to increase accuracy of prevalence estimates (**Appendix Tables 2** and **8** contain necropsy sample sizes for each species). All mass and lifespan data were log-transformed for statistical analysis due to their wide variation

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within the groups. We used generalized estimating equations (*compar.gee*) to estimate the influence of body mass or lifespan on neoplasia, cancer, and malignancy prevalence while taking into account the phylogenetic relationships within each group (Paradis et al., 2004; Paradis & Claude, 2002). As cancer prevalence is the response variable and is a proportion, the binomial family was used for the model.

Phylogenetic trees for each studied group were obtained from the following sources: amphibians (Alexander Pyron & Wiens, 2011)**,** birds (Jetz et al., 2012), crocodilians (Groh et al., 2020)**,** mammals (Upham et al., 2019)**,** squamates (Pyron et al., 2013)**,** and turtles (Thomson & Shaffer, 2010)**.** All the statistical analyses were run on R v4.1.1 (R Core Team, 2018)

RESULTS

Necropsies distribution

Our curated dataset included 7,691 necropsy reports from 604 species (626 species with Duke et al. (2022) squamate data). Out of all species known to exist in each group, our sampling represents 0.44% of all amphibians, 1.8% of all birds, 30.4% of all crocodilians, 3.6% of all mammals, 1.1% of all squamates, and 16.7% of all turtles (**Table 1**). Based on the Shannon-Wiener Diversity Index, birds and mammals had the highest sampled species diversity, but they also had the most necropsies, while crocodilians were underrepresented both in terms of number of species and number of necropsies compared to other tetrapod groups (**Table 1**). Even though the total number of species and individuals for each group was different, the Shannon Equitability Index indicated that the allocation of necropsies among species was similar within each of the groups (0.8-0.9, **Table 1**). Since species with fewer necropsies may be less reliable, we also calculated the percentage of species with ≥5 and ≥10 necropsies. Out of the total dataset, species with ≥5 necropsies comprised 44% of amphibians, 51% of birds, 29%

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of crocodilians, 63% of mammals, 38% of squamates (57% with Duke et al. (2022) data), and 20% of turtles in our dataset (**Appendix Table 2**). Species with ≥10 necropsies comprised 34% of birds, 39% of mammals, and 31% of squamates (14% without Duke et al. (2022) data) (**Appendix Table 8**).

Prevalence of *Neoplasia, Cancer, and Malignancy*

We found clear differences in prevalence of neoplasia, cancer, and malignancy among groups (**Figure 1**). Crocodilians and turtles had no instances of cancer, and thus malignancy, and only one case of neoplasia each, resulting in neoplasia prevalence values of 5% and 0.6%, respectively (**Table 1**). However, the number of species in these two groups with ≥5 necropsies per species was lower than the other groups (**Figure 2**; **Appendix Table 2**); specifically, in crocodilians, only two out of seven species (**Table 1**) had ≥5 necropsies. In contrast, birds were highly represented with 2,700 necropsy reports, yet their neoplasia prevalence was also low at 2%. Increasing the number of necropsies per species in crocodilians and turtles could increase the chance of detecting neoplasia and cancer, but each group also had several species with multiple necropsies in which neoplasia or cancer were not detected (**Figure 2**).

Within our dataset, neoplasia prevalence ranked from highest to lowest as follows: 10% in squamates (9% with Duke et al. (2022) data), 9% in mammals, 6% in amphibians, 5% in crocodilians, 2% in birds, and 0.6% in turtles (**Table 1**). This rank order did not change for cancer prevalence except that both crocodilians and turtles had zero prevalence. The malignancy prevalence (i.e., cancer divided by neoplasia counts) was highest in squamates (60%; 80% with Duke et al. (2022) data) and mammals (60%), then amphibians and birds (both 50%), and no instances of cancer in crocodilians and turtles (0%) (**Table 1**). Examining only species with ≥5 necropsies per species changed the values slightly but did not affect these rankings, except that amphibians had a 60% malignancy prevalence putting them above birds and on par with mammals and squamates (**Appendix Table 2**). The observed prevalence of

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neoplasia, cancer, and malignancy, and their 95% confidence intervals, among tetrapod groups indicate that birds and turtles have much lower neoplasia and cancer than other groups, but that birds are on par with other groups for malignancy (**Figure 3**). Pairwise comparisons of proportion of cancer, neoplasia, and malignancy prevalence calculated using *prop.test* further support these differences among groups. The three groups at the lower end of neoplasia prevalence (birds, crocodilians, and turtles) were not significantly different from each other for neoplasia or cancer prevalence (**Appendix Table 3**). Groups with higher proportions of neoplasia, cancer, and malignancy prevalence also do not significantly differ one from another (**Appendix Table 3**). Birds and turtles were significantly ($p<0.05$) different from mammals, squamates, and amphibians for neoplasia and cancer prevalence (**Appendix Table 3**). These trends were supported when the data was curated to only species with ≥5 necropsies except that turtle and amphibian neoplasia prevalence values were no longer significantly different from each other (**Appendix Table 4**).

Within each group, we found wide variation in cancer and neoplasia prevalence across families (**Appendix Table 5**). In amphibians, Pipidae had the highest neoplasia prevalence (50%) and was significantly ($p<0.05$) different from all other families (*Dryad after manuscript acceptance*). In turtles and crocodilians, the only neoplasms were found in Chelidae and Alligatoridae, respectively. In squamates, the highest neoplasia prevalence was found in the Helodermatidae (43%) and was significantly ($p<0.05$) different from all other families. For malignancy prevalence, two snake families, Colubridae and Viperidae (70% and 100%, respectively), were found to be significantly different from the lizard family Agamidae (40%) (*Dryad after manuscript acceptance*). In mammals, we found that cancer was widespread across orders, with 12 out of 17 orders represented in our work experiencing neoplasia and cancer (**Appendix Table 5**). We found very high prevalence of neoplasia and malignancy (61% and 88%, respectively) in Didelphimorphia, represented only by the Virginia opossum (*D.*

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virginiana) in our dataset, in Proboscidea (43% neoplasia and 44% malignancy), and Dasyuromorphia (50% neoplasia and 80% malignancy), with significant pairwise comparisons against most other orders (*Dryad after manuscript acceptance*). Finally, in birds, although neoplasia was found to occur across several orders with low prevalence (**Appendix Table 5**), higher prevalence was found in Rheiformes (25%) and Cuculiformes (50%), with significant pairwise comparisons against most other orders (*Dryad after manuscript acceptance*), although both orders were only represented by 4 necropsies per order.

Intrinsic cancer risk versus cancer and neoplasia prevalence

Intrinsic cancer risk corresponds to the predicted risk of developing cancer based on a species lifespan and body mass (Vazquez & Lynch, 2021). Across tetrapods, we found ICR to be highly variable (**Figure 4**). Certain species showed extraordinarily high ICR values compared to other species in their groups, including Chinese giant salamanders (*Andrias davidianus*), common ostriches (*Struthio camelus*), African bush elephants (*Loxodonta africana*), Komodo dragons (*Varanus komodoensis*), and Galapagos tortoises (*Geochelone nigra* complex). Overall, based on the species in our dataset, amphibians and turtles have lower average ICR values than other tetrapod groups (**Figure 4**).

Using phylogenetic comparative methods (*gee*), we found that the observed neoplasia and cancer prevalence in our dataset were influenced by estimated ICR in birds, mammals, and squamates (**Appendix Table 6**). Thus, when body mass and lifespan were combined as a single ICR value, it was a good predictor of neoplasia and cancer prevalence for most tetrapod groups. In mammals, there was a significant ($p<0.05$) negative relationship between ICR and cancer prevalence, indicating that larger, longer-lived species have lower rates of cancer. Conversely, there was a significant ($p<0.05$) positive relationship between ICR and cancer prevalence in birds and squamates, indicating that larger, longer-lived individuals have higher rates of cancer.

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Cancer and neoplasia prevalence as a function of body mass and lifespan

We tested the influence of body mass and lifespan separately on the prevalence of neoplasia, cancer, and malignancy in each group, while considering phylogenetic relationships among species in our dataset for each group. For groups with ≥70 species and ≥5 necropsies per species, we found that body mass significantly influenced neoplasia in birds and squamates (p=0.008 and p=0.02, respectively), with an increase in neoplasia with an increase in body mass (0.34 and 0.2 for direction of the slope, respectively, **Table 2**), and that lifespan influenced neoplasia in mammals (p=0.005) (**Table 2**). We also found that cancer prevalence was influenced by both body mass and lifespan in mammals (p=0.002 and p=0.02, respectively), although an increase in body mass or longevity does not reflect an increase in neoplasia or cancer prevalence (-0.23 and -0.62 for direction of the slope, respectively, **Table 2**). When analyses were run on the entire dataset for each group or species with ≥10 necropsies, these results were largely confirmed (**Appendix Tables 7** and **8**); the only exception was that malignancy (but not cancer) was dependent on body mass in mammals (p=0.008, **Appendix Table 8**). For mammals, when *gee* analyses were repeated using only the Boddy, et al. (2020a) dataset, we found that mass and lifespan did not influence neoplasia, cancer, or malignancy prevalence, supporting what was previously found by the authors using a different statistical approach.

DISCUSSION

We estimated neoplasia and cancer prevalence across amphibians, birds, crocodilians, mammals, squamates, and turtles focusing on variation both within and among groups. Previous studies explored cancer prevalence in birds and reptiles (sauropsids), but considered these lineages as a single taxon (Harris, 2022). Here, we analyzed cancer prevalence data in

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birds and individual groups of non-avian reptiles separately, unmasking important taxonomic variation across major tetrapod lineages (Chiari et al., 2018). Our dataset included a minimum of 20-64% of species per group that were represented by ≥5 necropsies per species, increasing the probability of detecting neoplasia and cancer in these species, and representing the largest dataset used so far for squamates.

We found that prevalence of neoplasia, cancer, and malignancy was higher in squamates than in other groups, especially in lizards with larger than average body sizes such as Helodermatidae (e.g., gila monster). In snakes, we also found a high prevalence of malignancy (above 70%) for all the families represented by several necropsies (5 out of 6 families). In contrast, we found no occurrence of cancer and only one species affected by neoplasia in both turtles (*Chelus fimbriata*) and crocodilians (*Alligator mississippiensis*). Turtles have lower mutation rates in mitochondrial and nuclear DNA (Lourenço et al., 2013) than mammals and birds, which may be related to the lower incidence of cancer found in these animals overall. In addition, turtles were recently found to have slower rates of aging and potential cellular mechanisms underlying delayed aging and cancer resistance (da Silva et al., 2022; Glaberman et al., 2021; Reinke et al., 2022). Growths and cancers do occur and can be detected in turtles and crocodilians (Garner et al., 2004; Sykes & Trupkiewicz, 2006). In previous work where neoplasia prevalence was not analyzed separately as benign or malignant, turtles were found to have between 1.2 and 2.7% neoplasia prevalence (Garner et al., 2004; Sykes & Trupkiewicz, 2006), while crocodilians were found to have a 2.2% neoplasia prevalence (Garner et al., 2004).

Our data also identified a low occurrence of neoplasia and cancer in birds, as previously observed for tumors in general (without distinction between benign and malignant) in comparison to mammals and reptiles in general (Effron et al., 1977; Madsen et al., 2017). The low prevalence of neoplasia and cancer in birds has been proposed to be due to enhanced immunity and the presence of the bursa of Fabricius (Møller et al., 2017), a specialized organ

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that produces B-cells in birds. However, our data indicated that although birds have very low prevalence of neoplasia, malignancy prevalence is similar to what is observed for mammals and squamates, to suggest that when neoplasia occurs in birds, this often (~50%) results in malignancy.

Previous studies have found conflicting results across groups regarding the influence of life history traits such as body mass and lifespan on prevalence of neoplasia and cancer. As was previously observed for birds (Møller et al., 2017), we found that body mass influences neoplasia prevalence. However, we establish a completely novel finding that squamates show this same positive relationship between body mass and neoplasia prevalence. Thus, in both birds and squamates, Peto's paradox is rejected, as species with higher body mass also have higher prevalence of neoplasia.

In mammals, cancer prevalence was previously found to be unrelated to body mass or lifespan but instead potentially influenced by diet, with higher prevalence of cancer seen in carnivores (Effron et al., 1977; Madsen et al., 2017; Vincze et al., 2022). However, since prevalence data are proportional, we used a more accurate statistical approach than previous studies, which treated prevalence in phylogenetic comparative analysis as a continuous variable (e.g., Phylogenetic Generalized Least Squares - PGLS - was used in Boddy et al., 2020a; a modified approach including PGLS was used in Vincze et al., 2022). Instead, the *gee* approach we used can handle various types of responses, including those expected to follow the binomial distribution, as for prevalence data (Paradis and Claude, 2002). Contrary to previous studies, we found that both body size and lifespan do in fact influence cancer and malignancy prevalence in this group, but in an inverse manner (i.e., increased lifespan and body mass is associated with lower cancer prevalence). These results suggest that larger, longer-lived species must have evolved mechanisms to reduce cancer risk below their smaller, shorter-lived counterparts. We did not find this pattern when we reanalyzed the mammalian dataset from Boddy et al. (2020a), possibly because the phylogenetic breadth and sample size was greater in

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our dataset. However, a recent study with 191 mammal species based on ≥20 necropsies per species did not find an influence of body mass and longevity on cancer (Vincze et al., 2022), possibly due to the use of life expectancy instead of maximum lifespan as done in our work.

Our results clearly indicate that the goal of identifying species that defy cancer risk predictions and have evolved strategies for cancer resistance requires larger datasets and appropriate analysis methods to accurately assess factors influencing cancer prevalence. Furthermore, our data show that, beyond mammals, other vertebrates likely hold novel insights into the evolution of cancer suppression. We found that Peto's Paradox is supported in mammals, but not in other vertebrates. Moreover, even if Peto's paradox occurs in mammals, lifespan and mass are inversely related to cancer prevalence. Finally, our results further support the wide variation in cancer prevalence within and among species and vertebrate groups. Further research is needed to identify why groups such as turtles and crocodilians have extremely low neoplasia and cancer incidence and why within certain groups, such as mammals, cancer does not occur in certain species as frequently as predicted by expectations.

ACKNOWLEDGMENTS

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TABLES

Table 1: Total number of necropsies and species representation for each group from the final curated dataset. The total recognized species number for each group was obtained from IUCN (*The IUCN Red List of Threatened Species*, 2022.). "Total neoplasia #" includes all benign and malignant tumor counts whereas "Total cancer #" includes only those tumors that were diagnosed as a malignancy by a veterinary pathologist and confirmed by histology. Malignancy prevalence is derived from the cancer count out of neoplasia count.

	Amphibians	Birds	Crocodilians	Mammals	Squamates	Squamates*	Turtles
# Species in dataset	32	204	7	213	103	125	45
Recognized # of species in group	7296	11162	23	5968	9855	9855	269
% of species represented	0.44	1.8	30.4	3.6	1.1	1.3	16.7
# of necropsies	180	2700	21	2804	557	1828	158
Shannon-Wiener Diversity Index	3.01	4.50	1.73	4.76	4.03	3.9	3.40
Shannon Equitability Index	0.868	0.844	0.888	0.89	0.870	0.822	0.894
Total neoplasia #	10	42	1	260	58	156	1
Total "cancer" #	5	21	0	157	37	129	0
Neoplasia Prevalence	0.06	0.02	0.05	0.09	0.1	0.09	0.006
Cancer Prevalence	0.03	0.008	0	0.06	0.07	0.07	0
Malignancy Prevalence	0.5	0.5	0	0.6	0.6	0.8	0

*Includes data from Duke et. al (2022)

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Table 2: Influence of body mass or lifespan on cancer, neoplasia, or malignancy prevalence using only species with five or more necropsies. Analyses run on species with five or more necropsies on cancer, neoplasia, and malignancy using either lifespan or body mass as the predicting variable and taking into account group's phylogenetic relationships. Analyses were run using the generalized estimating equations (*gee*; see Materials and Methods for additional information). P-values <0.05 are in bold with slope parameters in parentheses to show direction effect. Lines represent models that did not run due to non-converging.

Vertebrate Group	Cancer		Total Neoplasia		Malignancy	
	Mass p-value (slope)	**Lifespan p-value (slope)**	**Mass p-value (slope)**	**Lifespan p-value (slope)**	**Mass p-value**	**Lifespan p-value**
Amphibians	0.12	0.5	0.3	0.95	0.52	—
Birds	**0.05 (0.317)**	0.09	**0.008 (0.34)**	0.21	0.99	0.93
Mammals	**0.002 (-0.23)**	**0.02 (-0.62)**	0.9	**0.005 (-0.63)**	—	—
Squamates	0.3	—	**0.008 (0.3)**	—	0.18	—
Squamates w/Duke et al. (2022)	0.4	—	**0.02 (0.2)**	—	0.3	0.4
Turtles*	—	—	0.9	0.9	—	—

*Crocodilians and Turtles could not be analyzed for cancer and malignancy prevalence, as their cancer counts were zero. Crocodilians also could not be analyzed for neoplasia as only two species out of seven had 5 or more necropsies.

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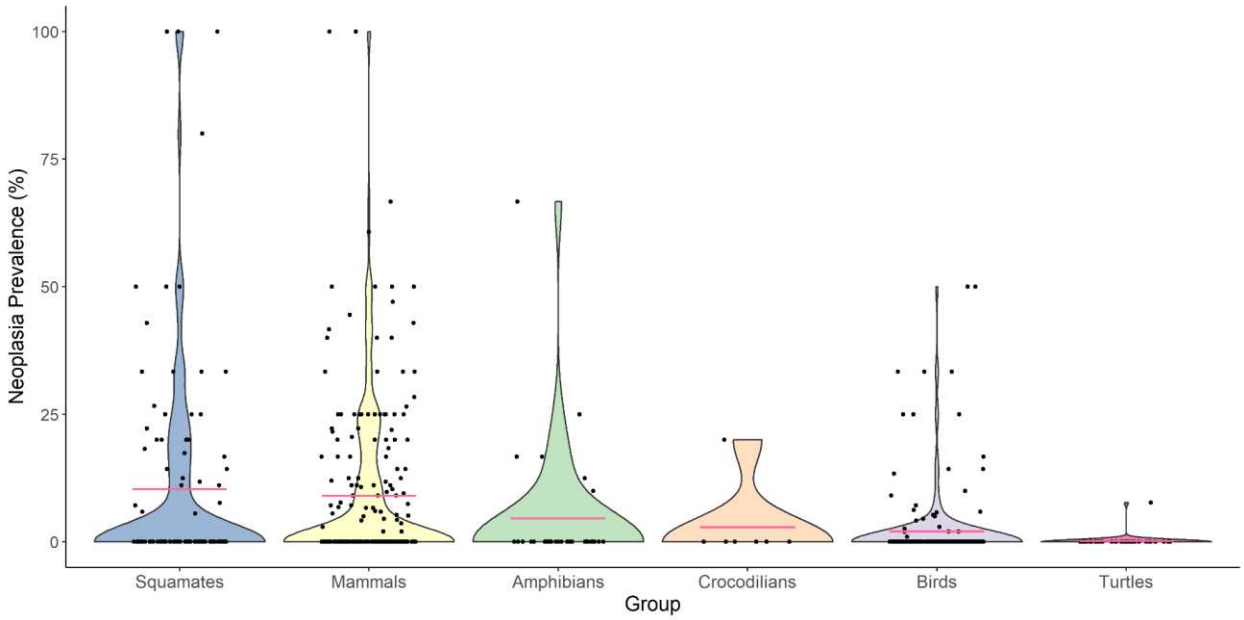
FIGURES

Figure 1. Summary of (a) neoplasia prevalence and (b) cancer prevalence. Each point represents the prevalence of a single species. Each red line represents the mean of the group.

(a)



(b)

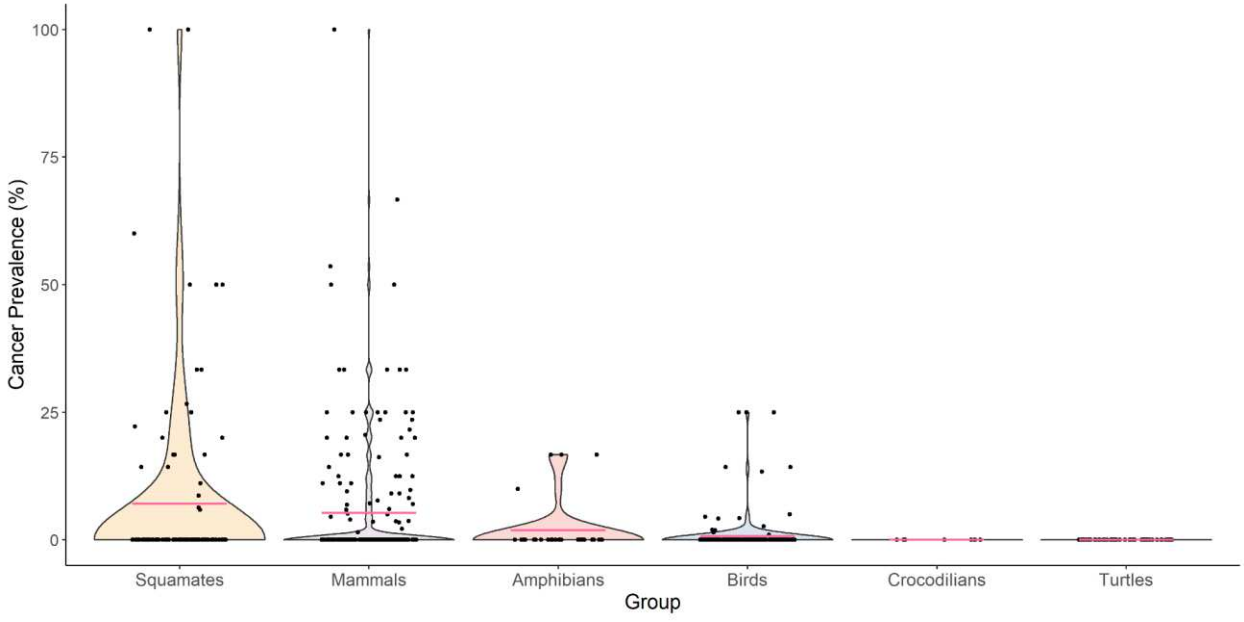


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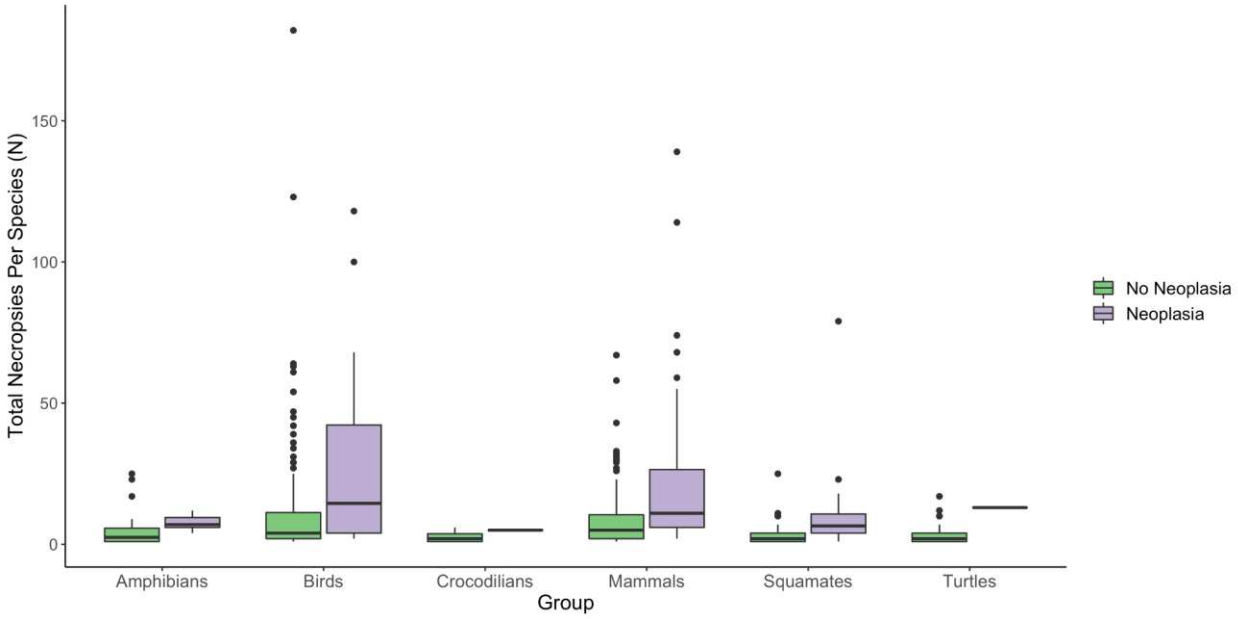
Figure 2. Presence or absence of (a) neoplasia or (b) cancer as a function of total number of necropsies per species across tetrapod groups.

(a)



(b)

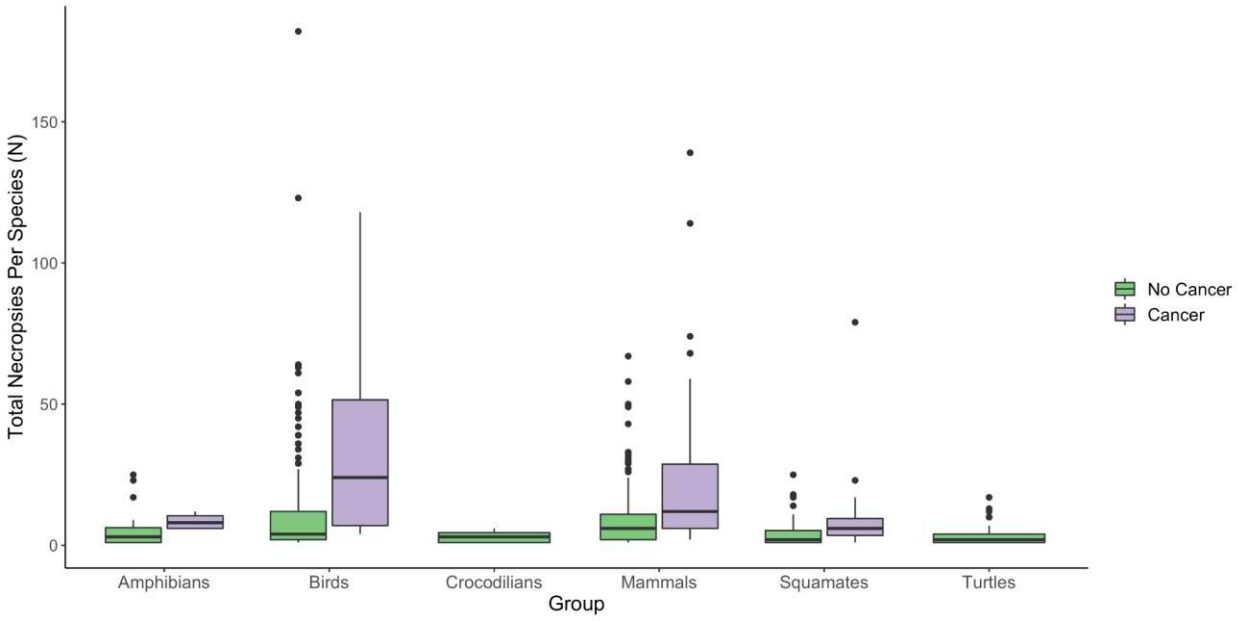


Figure 3. Prevalence of neoplasia, cancer, and malignancy across tetrapod groups. Points represent observed prevalence values and lines represent the 95% confidence intervals surrounding the observed prevalence value. * Indicates squamate dataset using data from Duke et al. (2022).

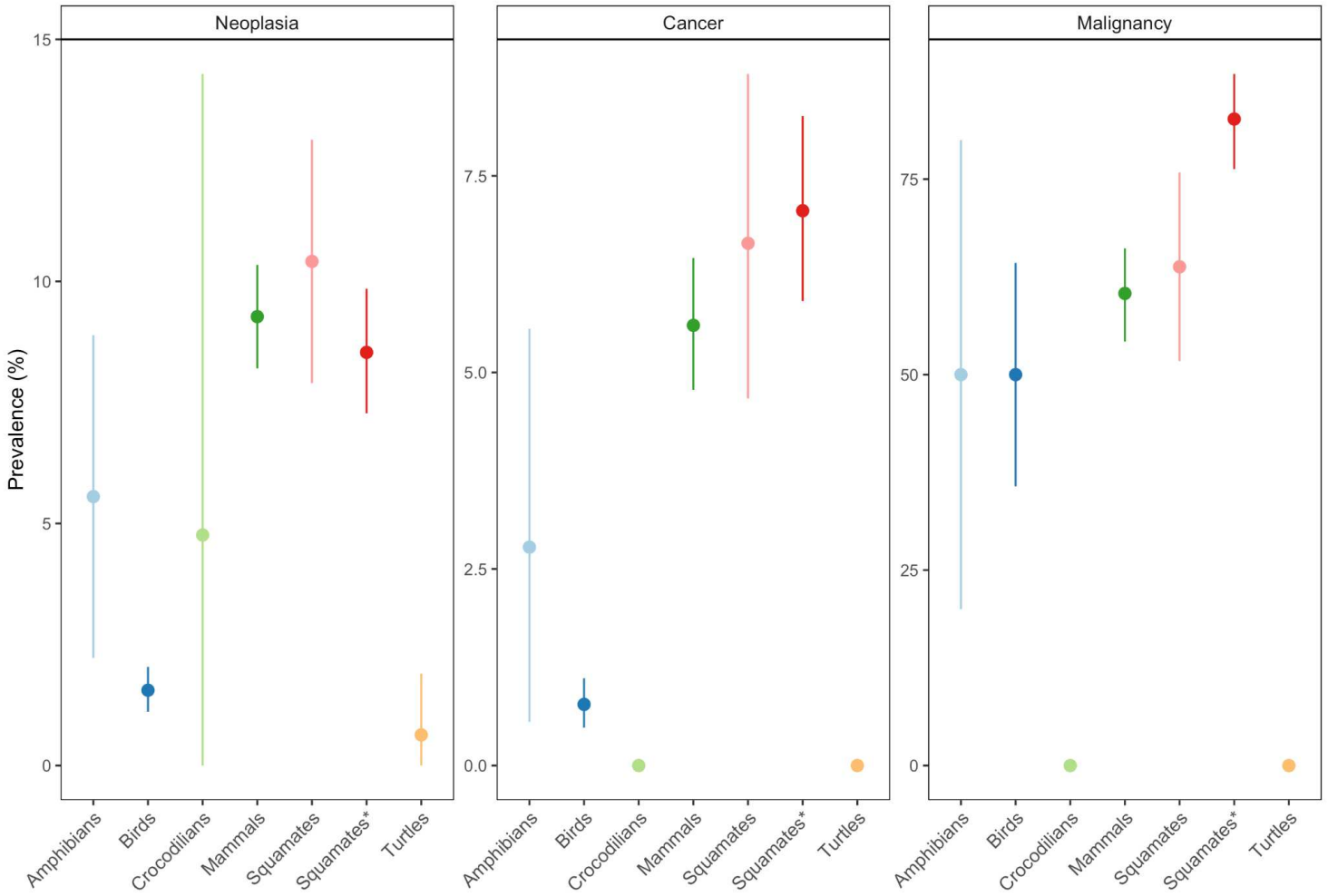


Figure 4. Estimated intrinsic cancer rates (ICR) based on body mass and lifespan data for all tetrapod species examined in this study (see Materials and Methods for ICR calculation). Gray lines in each violin represent the mean ICR for the tetrapod group.