Cancer prevalence is related to body mass and lifespan in tetrapods and remarkably low in turtles

Short title: **Cancer prevalence across tetrapods**

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ABSTRACT

Identifying species with lower-than-expected cancer prevalence can help establish new models for understanding cancer resistance. Most studies of cancer prevalence have focused on mammals. Yet, other vertebrate groups vary tremendously in genetics, physiology, and ecology, which can all influence mechanisms of cancer resistance and may hold new keys to understanding cancer biology. Here, we present data on cancer prevalence in tetrapods, which includes all major vertebrate groups except fish, using necropsies from over a thousand different species. We investigated cancer prevalence within and among amphibians, birds, crocodilians, mammals, squamates, and turtles in relationship to body mass and lifespan. We are the first to (1) analyze non-avian reptile groups separately, (2) conduct statistical analyses appropriate for this type of discontinuous data, (3) more accurately use raw cancer occurrence data instead of species averages, and (4) look at how data resampling influences the robustness of results. We found remarkably low cancer prevalence in turtles and high prevalence in squamates and mammals. In contrast to previous studies, lifespan in mammals was negatively associated with neoplasia but positively associated with malignancy. We recovered the same results by reanalyzing data from these previous studies with our statistical approach. We also found that neoplasia prevalence was positively associated with body mass in amphibians and squamates. Overall, our results demonstrate a clear relationship between life history and neoplasia in most tetrapod groups. Our findings also indicate that choice of analytical methods is critical when examining cancer prevalence data. Finally, the exceptionally low cancer prevalence in turtles and extensive variation in cancer prevalence among tetrapod families hold particular promise for identifying species with novel mechanisms of cancer resistance.

KEYWORDS: Life history, neoplasia, Peto's paradox, cancer prevalence, zoo, comparative oncology, vertebrates

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; Kitsoulis et al., 2020; 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, Schiffman, et al., 2017; Tollis, Boddy, et al., 2017). Consistent with these expectations, cancer prevalence increases with both body size and age within certain species such as humans and dogs (Dobson, 2013; Nunney, 2018). However, previous 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; Kapsetaki et al., 2023) – a phenomenon known as Peto's paradox (Nunney et al., 2015; Peto et al., 1975). To explain this seeming contradiction, animals with extreme body sizes and lifespans must have developed physiological mechanisms that offset their increased cancer risk (Caulin & Maley, 2011; Nery et al., 2022). Therefore, examining variation in cancer prevalence within and between vertebrate groups may 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 (Compton et al., 2023; 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), with ferrets and opossums having remarkably higher cancer prevalence compared to other mammal species (Boddy et al.,

2020a: Compton et al., 2023). Among non-avian reptiles, turtles appear to have lower prevalence than squamates (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 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, especially for groups outside mammals (Compton et al., 2023; Harris, 2022; but see Kapsetaki et al., 2023 for birds). 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 and among major vertebrate taxa makes it easier to identify cellular, physiological, and ecological drivers of cancer prevalence across animals.

Here we use necropsy data, including neoplasia and cancer incidence, from amphibians, birds, crocodilians, mammals, squamates, and turtles from multiple zoos, in order to compare cancer prevalence within and between tetrapods, which includes all major vertebrate groups except fish. We 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), in contrast to previous studies that have typically treated prevalence as continuous (e.g., Boddy et al., 2020a; Compton et al., 2023; Kapsetaki et al., 2023). Unlike previous studies, we use the raw data for each species instead of species averages in order to more effectively account for intraspecific sampling effort. Finally, we critically analyze our data in comparison to all previous publications on mammals (Boddy, Abegglen, et al., 2020; Vincze et al., 2022) by using jackknife to confirm the reliability of our findings and conclusions and assess the influence of intraspecific sampling effort on the results. Our novel approach shows that neoplasia and cancer prevalence vary tremendously within and among vertebrate groups and,

unlike previous studies, are influenced by body mass and lifespan in amphibians, squamates, and mammals.

MATERIALS AND METHODS

Cancer Data Collection and Curation

We searched paper reports or digital databases of six European zoos (Allwetterzoo, Münster, Germany; ZOOM Erlebniswelt Gelsenkirchen, Gelsenkirchen, Germany; Der Grüner Zoo Wuppertal, Wuppertal, Germany; Wildlife Zoo Hellenthal, Hellenthal, Germany; Rotterdam Zoo, Rotterdam, The Netherlands; Zoo Zürich, Zürich, Switzerland) and one in the United States (Birmingham Zoo, Alabama) for necropsies recorded between 1998 and 2019 (**Appendix Table 1**). The databases for Allwetterzoo, ZOOM Erlebniswelt, Zoo Wuppertal, Zoo Hellenthal, Zürich Zoo, 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 used 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 to search for 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 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. 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 analyses.

“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

Maximum body mass and lifespan were collected for each species when available using the Animal Aging and Longevity Database (AnAge) (Tacutu et al., 2018). If no species data was found in AnAge, primary sources were used when available. Data origin for 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 life history trait analyses. Most amphibian life history trait data were collected 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 = $\text{lifespan}^6 \times \text{body mass}$. 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 and 1 represent the species with the lowest and highest intrinsic cancer risk, respectively, within each lineage. To test the relationship between expected cancer risk and observed neoplasia, cancer, and malignancy prevalence for each group, while taking phylogenetic relationships into account, 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

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). Additional information on Methods and Results for this can be found in the Appendix (**Appendix Table 2**).

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 our knowledge, this is the first work taking into account confidence intervals for estimates of cancer prevalence within each group, thus assessing whether we have sufficient data for each group to generate reliable estimates.

To assess the influence of lifespan and body mass on neoplasia, cancer, and malignancy, statistical analyses were run for neoplasia prevalence, cancer prevalence, and malignancy prevalence. Analyses were run for the entire dataset and also considering species with ≥5 or ≥10 necropsies per species (**Appendix Tables 3** and **4** contain necropsy sample sizes for each species). The rationale behind repeating the analyses with the full dataset and only species with more necropsies is that, in the former case (i.e., more species), phylogenetic resolution is higher and the data distribution across the phylogeny is not biased towards an overrepresentation of data for certain clades, which could produce misleading results or non-convergence in the analyses. On the other hand, using only species with a higher number of necropsies may increase accuracy of prevalence estimates – especially for species where instances of cancer are rare – as done by others in similar studies (Boddy et al., 2020a; Vincze et al., 2022).

In order to obtain an idea of the influence of interspecific sampling within groups, analyses were run by jackknifing species of the dataset and a 95% confidence interval was

obtained for the estimated parameters. For the statistical analyses, mass and lifespan data were log-transformed to linearize potential allometric effects. 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 and the number of positive (occurrence of neoplasia, cancer) and negative (non-occurrence) individuals was entered as raw data for each species. For these comparative analyses in which phylogenetic relationships and each life history trait was analyzed, we only used species that could be matched with a published phylogeny for their group and for which at least one life history trait (lifespan and/or body mass) could be found online or in literature (see Life History Data Collection). The novelty of this approach is that instead of using proportions for neoplasia, cancer, or malignancy per species, as commonly done (Boddy, Abegglen, et al., 2020; Compton et al., 2023; Kapsetaki et al., 2023; Vincze et al., 2022), we used individual data for each species. This ensures that per species sample size is taken into account directly in the model, without the need of giving different weights according to sampling; this also helps to directly model the prevalence as a logistic response. Numbers of species per group for all analyses can be found in **Table 2** and in the Appendix (**Tables 3** and **4**). These analyses were not run for the crocodilians as there were too few species to allow convergence of 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 phylogenetic trees were transformed to ultrametric trees using the *chronos* function in R package [ape], using default parameters (no fossil calibration). All the statistical analyses were run on R v4.1.1 (R Core Team, 2018).

Comparison With Previously Published Results On Mammals

To compare our results with what has previously been reported for mammals, we reanalyzed the data from Boddy et al. (2020a) and Vincze et al. (2022), separately, using *gee*. Body mass and lifespan data were log transformed as the authors did in their papers. For the Vincze et al. (2022) data, we used the estimates of life expectancy data calculated by the authors instead of maximum lifespan for species, following what the authors did in their paper. Vincze et al. also had only one category for neoplastic conditions represented by any neoplasm that was diagnosed as cause of death while Boddy et al. (2020a) separated total neoplasms and cancer as in our work. As such, the data of Vincze et al. (2022) could only be analyzed for neoplasia prevalence, while the data of Boddy et al. (2020a) were analyzed for neoplasia, cancer, and malignancy prevalence.

RESULTS

Necropsy Data

Our curated dataset included 9,631 necropsy reports from 1,030 species, or 1,060 species including Duke et al. (2022) squamate data. Out of the total described species in each tetrapod group, our sampling represents 0.7% of all amphibians, 4.5% of all birds, 34.7% of all crocodilians, 4.0% of all mammals, 1.8% of all squamates, and 21.0% of all turtles (**Table 1**). The Shannon Equitability Index indicated that the allocation of necropsies among species was similar within each group (**Appendix Table 2**).

Prevalence of Neoplasia, Cancer, and Malignancy

We found clear differences among groups (**Figures 1, 2,** and **3**), with certain groups having especially low prevalence of neoplasia, cancer, and/or malignancy. Turtles had no instances of cancer, and thus malignancy, and only one case of neoplasia. Crocodilians had only two neoplasias, one of which was diagnosed as malignant (**Table 1**). Birds were highly

represented in our dataset, with 507 species and 5,121 necropsy reports. Neoplasia and cancer prevalence values were particularly low in this group, at 2% and 1%, respectively, while malignancy prevalence was 60% (**Table 1**). Having more necropsies per species increased the chance of detecting neoplasia and cancer (**Appendix Figure 1**), although each tetrapod group had species with multiple necropsies in which neoplasia or cancer were not detected.

Within our dataset, neoplasia prevalence ranked from highest to lowest as follows: 8% in both squamates and crocodilians, 7% in mammals, 4% in amphibians, 2% in birds, and 0.5% in turtles (**Table 1** and **Figure 2**). Cancer prevalence ranked from highest to lowest as follows: 5% in squamates, 4% in both mammals and crocodilians, 3% in amphibians, 1% in birds, and 0% in turtles. Malignancy prevalence (i.e., cancer divided by neoplasia) was highest in squamates and amphibians with both groups having 70% malignancy rates (80% malignancy in squamates including Duke et al., 2022 data), followed by mammals and birds with 60%, and crocodilians with 50% (**Table 1** and **Figure 2**). When considering species with ≥5 necropsies per species, squamates still had the highest prevalence of neoplasia and cancer, while amphibians and birds had higher malignancy than other groups (**Appendix Table 3**). The observed prevalence values and their 95% confidence intervals among tetrapod groups indicated that birds and turtles had much lower neoplasia and cancer prevalence than other groups, but that birds were similar to other groups for malignancy (**Figure 2**). Except for crocodilian and amphibian cancer and malignancy, 95% confidence intervals for prevalence values were narrow (**Figure 2**), indicating that estimates were precise.

Pairwise comparisons of neoplasia, cancer, and malignancy prevalence calculated using *prop.test* further supported differences among tetrapod groups (**Table 2**). The two groups at the lower end of neoplasia and cancer prevalence (birds and turtles) were not significantly different from each other, but were different from all the other groups (except birds from crocodilians **Table 2**). For neoplasia, amphibians differed from mammals and squamates, which had the highest neoplasia prevalence. Amphibians and squamates also differed from each other for

cancer prevalence, but not for malignancy (**Table 2**). 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 5**).

Within each tetrapod group, we found wide variation in neoplasia and cancer prevalence among families (**Figure 3, Appendix Table 6,** full comparisons on **Dryad**). In amphibians, Pipidae (30%) and Ambystomatidae (27%) had the highest neoplasia prevalence values, which were significantly ($p<0.05$) different from other major amphibian families including Bufonidae, Dendrobatidae, Hylidae, Microhylidae, and Salamandridae. Ten amphibian families had 0% neoplasia prevalence, and Bufonidae had the lowest non-zero prevalence (3%). In turtles, the only neoplasm was found in *Chelus fimbriata* (Chelidae). In crocodilians, there were only two incidences of neoplasia, one each in Alligatoridae and Crocodylidae, with malignancy only occurring in *Osteolaemus tetraspis* (Crocodylidae). In squamates, the highest neoplasia prevalence was found in the Helodermatidae (25%) and was significantly ($p<0.05$) different from several other squamate families. Malignancy prevalence was high in snakes, especially in the Colubridae (70%) and Viperidae (100%). Cancer was widespread across mammals, with 10 out of 17 orders represented in our work experiencing neoplasia and cancer (**Appendix Table 6**). The highest prevalence values occurred in carnivores (15% neoplasia and 80% malignancy) and primates (11% neoplasia and 48% malignancy). In the majority of cases in which neoplasia was detected in mammals, malignancy prevalence was also high (>30%) with the exception of Chiroptera (4% neoplasia and 0% malignancy) and Proboscidea (25% neoplasia and 0% malignancy). Finally, in birds, although neoplasia was found to occur across several orders with low prevalence (**Appendix Table 6**), higher neoplasia prevalence was found in Cuculiformes (18.2%), with significant pairwise differences from most other avian orders. When malignancy did occur in birds, the prevalence was extremely high (>50%), with the exception of Pelecaniformes (40%) and Phoenicopteriformes (33%).

Intrinsic Cancer Risk Versus Neoplasia and Cancer Prevalence

Intrinsic cancer risk corresponds to the predicted risk of developing cancer based on a combination of species lifespan and body mass (Peto, 2015; 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*), Asian elephants (*Elaphas maximus*), Komodo dragons (*Varanus komodoensis*), and Galapagos tortoises (*Geochelone nigra* complex). Overall, based on the species in our dataset, amphibians and turtles had lower average ICR values than other tetrapod groups (**Figure 4**).

Using phylogenetic comparative methods (*gee*), we found a significant (p=0.007) positive relationship between neoplasia prevalence and estimated ICR in squamates, and a significant (p= 0.0002) negative relationship for mammals (**Appendix Table 7**). We also found that cancer prevalence was positively associated with ICR values in amphibians (p=0.002; **Appendix Table 7**). Thus, ICR is a good predictor of neoplasia or cancer prevalence for squamates, mammals, and amphibians, but not for the other groups. ICR was not related to malignancy in any group.

Neoplasia And Cancer Prevalence As A Function Of Body Mass And Lifespan

We also tested the influence of body mass and lifespan separately on the prevalence of neoplasia, cancer, and malignancy in each tetrapod group, while accounting for phylogenetic relationships among species within each group. We found that body mass was positively correlated with neoplasia and cancer in amphibians, neoplasia in squamates, and neoplasia and malignancy in mammals (**Table 3**). We found that lifespan was positively correlated to cancer in amphibians, neoplasia and cancer in squamates, and malignancy in mammals (**Table 3**). A positive correlation means that increases in that particular life history trait correspond to higher prevalence, which supports our predictions using ICR (**Appendix Table 7**). On the other hand,

in mammals, we found a negative relationship between body mass and lifespan and neoplasia, as well as body mass and malignancy, meaning that increases in that life history trait correspond to lower prevalence (**Table 3**). Jackknife analyses indicated that our full dataset produces reliable results, as 95% confidence intervals never included 0 when tests were significant and became narrower when larger numbers of species were sampled (**Table 3**).

For groups with ≥70 species and ≥5 necropsies per species (birds, mammals, and squamates), we found that when analyses converged, they largely confirmed the results obtained with the full dataset (**Appendix Table 8**). For groups with ≥70 species and ≥10 necropsies per species (birds, mammals), the only differences in results were that the relationship between lifespan and cancer in birds became significant and body mass no longer significantly influenced malignancy or neoplasia in mammals (**Appendix Table 8**).

Comparison of Results With Previous Studies On Mammals

Previous studies in mammals (Boddy et al., 2020; Vincze et al., 2022) have found that body mass and lifespan do not influence neoplasia and cancer prevalence (but see Compton et al., 2023 on the influence of body weight after correcting for gestational period). To directly compare our results with prior studies, we re-analyzed their data using our statistical approach (*gee*). Based on the data from Boddy et al. (2020), which included 36 species (28 of them with ≥10 necropsies per species), we found no influence of body mass or lifespan on neoplasia or cancer prevalence, supporting their results (**Appendix Table 9**). However, as their interspecific sampling is lower than ours and as parameter estimates were close to 0 (**Appendix Table 9**, see Jackknife values), it could also mean that their sample size is too low to detect relationships. However, when we used the data from Vincze et al. (2022), including 189 species with ≥19 necropsies per species, our statistical approach indicated that both body mass and life expectancy were significantly ($p<0.001$) related to neoplasia prevalence, in contrast to what was found by the authors. The fact that we found a significant relationship between life history traits

and neoplasia prevalence with the dataset of Vincze et al. (2022) independently confirms our results showing these same relationships with our own dataset (**Appendix Table 9**).

DISCUSSION

We estimated neoplasia, cancer, and malignancy prevalence across tetrapods, including amphibians, birds, crocodilians, mammals, squamates, and turtles, focusing on variation both within and among groups. Previous studies have explored cancer prevalence in birds and reptiles (sauropsids), but considered these lineages as a single taxon (Compton et al., 2023; Harris, 2022) or analyzed birds alone (Kapsetaki et al., 2023); (Kapsetaki et al., 2023; Møller et al., 2017). Here, we analyzed prevalence data in birds and individual groups of non-avian reptiles separately, revealing important taxonomic variation across major tetrapod lineages (Chiari et al., 2018). We also used 95% confidence intervals to evaluate the robustness of our results. We found that neoplasia, cancer, and malignancy prevalence values were higher in squamates than in other groups, and were especially high in lizards with larger than average body sizes such as the Helodermatidae (e.g., gila monster). We also found high malignancy prevalence almost uniformly across snakes (67-100% among families). In contrast, we found no instances of cancer and only one case of neoplasia (*Chelus fimbriata*) in our turtle dataset. In crocodilians, we only found two species with neoplasia, one of which was malignant (*Osteolaemus tetraspis*).

Despite the remarkably low prevalence values for these groups in our dataset, neoplasia and cancer do occur and can be detected in turtles and crocodilians (Chang et al., 2012; Garner et al., 2004; Madsen et al., 2017; Sykes & Trupkiewicz, 2006). Therefore, our results for these groups were not biased by misdiagnosis or failure to recognize cancers. In previous work, which did not distinguish between benign and malignant tumors, turtles had 1.2-2.7% neoplasia prevalence (Garner et al., 2004; Sykes & Trupkiewicz, 2006), while crocodilians had 2.2% neoplasia prevalence (Garner et al., 2004). Turtles have lower germline mutation rates in

mitochondrial and nuclear DNA than mammals and birds (Lourenço et al., 2013), which may be related to lower somatic mutation rates and a lower overall incidence of cancer. In addition, turtles were recently found to have slower rates of aging and derived cellular phenotypes that may be associated with delayed aging and cancer resistance (da Silva et al., 2022; Glaberman et al., 2021; Reinke et al., 2022).

We also identified low rates of neoplasia and cancer in birds in comparison to mammals and reptiles, which has been observed in previous studies that combined benign and malignant tumors (Effron et al., 1977; Madsen et al., 2017). The low prevalence of neoplasia and cancer in birds has been proposed to be related to the size of the bursa of Fabricius, a specialized organ that produces B-cells in juvenile birds, as well as enhanced immune system development (Møller et al., 2017). Higher prevalence of neoplasia and malignancy in some avian species has been correlated with larger clutch sizes (Kapsetaki et al., 2023). Our data confirmed that birds do have very low rates of neoplasia, but malignancy prevalence is similar to mammals and squamates. These results indicate that when neoplasia does occur in birds it is usually malignant (>50% of the time).

Previous studies have found conflicting results across tetrapod groups regarding the influence of life history traits such as body mass and lifespan on neoplasia and cancer prevalence. Overall, we showed that at least one of these two life history traits has an influence on neoplasia or cancer in most tetrapod groups including amphibians, squamates, and mammals. Conversely, a previous study observed a positive association between body mass and neoplasia prevalence in birds (Møller et al., 2017), but our results contradict this observation. In mammals, cancer prevalence was previously found to be unrelated to body mass or lifespan, but instead potentially influenced by diet, with higher prevalence values for cancer observed in carnivores (Efron et al., 2001; Madsen et al., 2017; Vincze et al., 2022), or by gestation period and litter size (Boddy, et al., 2020a; Compton et al., 2023). In contrast to previous studies, we found a positive relationship between lifespan and malignancy in

mammals, but inverse relationships between body mass and both neoplasia and malignancy as well as an inverse relationship between lifespan and neoplasia. This suggests that animals with larger body mass have less neoplasia and malignancy, but that a more complex relationship exists between lifespan, neoplasia, and malignancy. Longer-lived species may have evolved mechanisms protecting them from developing neoplasia, but once it occurs, it may often become malignant, hence the positive relationship with malignancy. In this work, we have established completely new findings that squamates and amphibians both have a positive relationship between body mass and neoplasia prevalence, and amphibians also have a positive relationship between body mass and cancer. Remarkably, we found that neoplasia, cancer, and malignancy are especially frequent in snakes and occur in almost all the snake families included in this study.

Previous studies have treated cancer prevalence data as a continuous variable and used phylogenetic comparative methods based on Phylogenetic Generalized Least Squares (PGLS) to infer whether life history traits are correlated with cancer prevalence (Boddy et al., 2020a; a modified PGLS approach in Vincze et al., 2022). However, prevalence data are proportional rather than continuous and follow a binomial distribution (Paradis and Claude, 2002); therefore, a more appropriate statistical approach than PGLS is necessary to analyze these data. The *gee* approach used in this work can handle various types of responses, including those expected to follow a binomial distribution as in the case of prevalence data, and it is therefore a more comprehensive way to perform this analysis. Furthermore, *gee* uses counts of neoplasia and cancer based on each individual necropsied rather than calculating a single prevalence value for each species on the phylogeny. Unlike PGLS, this approach accounts for per species sample sizes and the distribution of the individual data across the phylogeny in a single analysis. To assess whether our analytical approach may explain why our findings differ from previously published work, we reanalyzed the mammalian data from both Boddy et al (2020a) and Vincze et al. (2022) using our statistical methodology. We found that

when the Vincze et al.'s (2022) data were reanalyzed with our approach, their data corroborated our findings, which supports the influence of both body mass and lifespan on neoplasia. This suggests that a relationship between life history traits and neoplasia was not recovered due to the use of a less suitable statistical method.

Caveats And Future Directions

Our results clearly indicate an influence of body mass and lifespan on neoplasia and cancer across most tetrapod groups. Our results, and the subsequent jackknife analysis we performed, indicate that fewer data, the presence of long branches in the phylogeny of focal species, and a scattered distribution of neoplasia, cancer, or malignancy occurrence across the phylogeny may influence the results obtained with comparative phylogenetic methods. As such, larger datasets in terms of both species and necropsies per species may solve some issues associated with analyzing this type of data.

Conclusions

Our data show that different vertebrate groups likely hold unique insights into the evolution of cancer suppression. In mammals, we found support for Peto's Paradox, where selection acts to repress neoplasia in larger and/or long-lived species. Yet, we also found a positive relationship between malignancy and lifespan in mammals, which indicates that once a growth occurs it often becomes malignant. These patterns suggest that evolution has selected traits to reduce the development of neoplasia rather than cancer, which can help focus future efforts to understand mechanisms of cancer biology in mammals, including humans.

Beyond mammals, we found that body mass and lifespan are also good predictors of neoplasia prevalence in amphibians and squamates, but not malignancy. Contrastingly, in birds, relatively low rates of neoplasia and cancer indicate that this group as a whole has evolved mechanisms to resist neoplasia regardless of life history. Yet the high rate of malignancy in

birds indicates that once neoplasia occurs it often becomes malignant. Finally, turtles, and possibly crocodilians, are potentially promising models for studying cancer resistance as they are characterized by extremely low levels of neoplasia and even lower rate of malignancy.

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AUTHOR CONTRIBUTIONS

Conceptualization: YC, SG; Investigation: SB, LP, WA, NM, J-PA, SD, SMcC, PW, SB, LRD, LGRB - vanS, DF, SG, YC; Data curation: SB, SG, YC; Formal Analysis: SB, JC, SG, YC; Writing – Original Draft: SB, SG, YC; Writing – Review and Editing: SMcC, PW, LGRB - vanS, DF, VJL, JC.

COMPETING INTERESTS

Authors claim no competing interests.

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TABLES

Table 1. Total number of necropsies and species for each tetrapod group. The total recognized number of species for each group was obtained from IUCN (*The IUCN Red List of Threatened Species*, assessed 2022.). "Total neoplasia" includes all benign and malignant tumor counts whereas "Total cancer" includes only those tumors that were diagnosed as malignant by a veterinary pathologist and confirmed by histology. Malignancy prevalence was calculated as neoplasia count divided by cancer count.

	Amphibians	Birds	Crocodilians	Mammals	Squamates	Squamates*	Turtles
Species in dataset	49	507	8	230	179	209	57
Recognized species in group	7296	11162	23	5968	9855	9855	269
Percentage of recognized species represented	0.7	4.5	34.7	4.0	1.8	2.1	21.0
Total necropsies	383	5121	26	2841	1053	2608	207
Total neoplasia	15	96	2	191	84	198	1
Total cancer	10	61	1	111	57	164	0
Neoplasia prevalence	0.04	0.02	0.08	0.07	0.08	0.08	0.005
Cancer prevalence	0.03	0.01	0.04	0.04	0.05	0.06	0
Malignancy prevalence	0.7	0.6	0.5	0.6	0.7	0.8	0

*Includes data from Duke et. al (2022)

Table 2: Pairwise comparison results for (a) neoplasia, (b) cancer, and (c) malignancy prevalence proportions run on full dataset (as in Table 1) using *prop.test* (R package {stats}). Numbers in parentheses are proportion numbers per group calculated as # group neoplasia / # group total necropsy or # group cancer/ # group necropsy total, and # group cancer / # group total neoplasia for malignancy. Significant values are in bold. Malignancy could not be calculated for turtles since there were no instances of cancer in these groups. The asterisk denotes squamates data included from Duke et. al (2022).

(a)

GROUP NEOPLASIA PREVALENCE							
	Amphibians (15/383)	Birds (96/5124)	Crocodilians (2/25)	Mammals (191/2841)	Squamates (84/1053)	Squamates* (198/2608)	Turtles (1/207)
Turtles (1/207)	**0.03**	0.2	**0.03**	**6.00E-04**	**2.20E-16**	**2.00E-04**	1
Squamates* (198/2608)	**0.01**	**2.20E-16**	1	0.2	0.7	1	
Squamates (84/1053)	**0.01**	**2.20E-16**	1	0.2	1		
Mammals (191/2841)	**0.05**	**2.20E-16**	1	1			
Crocodilians (2/25)	0.6	0.1	1				
Birds (96/5124)	**0.01**	1					

(b)

GROUP CANCER PREVALENCE							
	Amphibians (10/383)	Birds (61/5124)	Crocodilians (1/25)	Mammals (111/2841)	Squamates (57/1053)	Squamates* (164/2608)	Turtles (0/207)
Turtles (0/207)	**2.20E-16**	0.2	0.2	**0.007**	**0.001**	**0.0004**	1
Squamates* (164/2608)	**0.006**	**2.20E-16**	1	**8.0E-5**	0.4	1	
Squamates (57/1053)	**0.04**	**2.20E-16**	1	**0.05**	1		

Mammals (111/2841)	0.3	**2.60E-15**	1	1			
Crocodilians (1/25)	1	0.7	1				
Birds (61/5124)	0.03	1					

(c)

GROUP MALIGNANCY PREVALENCE						
	Amphibians (10/15)	Birds (55/82)	Crocodilians (1/2)	Mammals (106/185)	Squamates (50/75)	Squamates* (140/173)
Squamates* (140/173)	0.2	**0.0004**	0.8	**1.60E-07**	**0.008**	1
Squamates (50/75)	1	0.7	1	0.2	1	
Mammals (106/185)	0.7	0.5	1	1		
Crocodilians (1/2)	1	1	1			
Birds (55/82)	1	1				

Table 3. Influence of body mass and lifespan on cancer, neoplasia, and malignancy prevalence. Analyses run using the full dataset (for each group this is represented with the numbers above the lines) and on species with five or more necropsies (represented for each group below the line) 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 and jackknife 95% confidence intervals italicized in brackets. Lines represent models that did not run due to non-converging (see Materials and Methods for additional information). Numbers in the column "N Species" refers to the number of species per trait being analyzed. If a species mass or lifespan history trait was unknown it was removed from that analysis. Crocodilians could not be analyzed due to an insufficient number of species. Turtles could not be analyzed for cancer and malignancy as no instances of cancer were found.

Vertebrate Group	Total Neoplasia				Cancer				Malignancy			
	Mass		Lifespan		Mass		Lifespan		Mass		Lifespan	
	N Species	p-value (slope) *[jack knife]*	N Species	p-value (slope) *[jack knife]*	N Species	p-value (slope) *[jack knife]*	N Species	p-value (slope) *[jack knife]*	N Species	p-value (slope) *[jack knife]*	N Species	p-value (slope) *[jack knife]*
Amphibians	47	**0.003 (1.6) *[1.3 , 2.1]***	42	0.31 (0.7) *[0.2 , 1.5]*	47	**0.0014 (1.5) *[1.3 , 2.3]***	42	**0.009 (1.8) *[1.1 , 2.2]***	8	0.85 (0.3) *[0.12 , 1.4]*	8	—
Birds	299	0.4 (0.07) *[0.07 , 0.1]*	267	0.3 (0.3) *[0.3 , 0.4]*	299	0.6 (0.07) *[0.06 , 0.08]*	267	0.96 (-0.02) *[0.03 , 0.08]*	57	0.12 (0.3) *[0.2 , 0.3]*	48	0.3 (-0.5) *[-0.7 , -0.4]*
Mammals	220	**0.002 (-0.2) *[-0.2 , -0.15]***	218	**0.001 (-0.8) *[-0.8 , -0.4]***	220	0.6 (0.03) *[-9e-4 , 0.05]*	218	0.3 (-0.3) *[-0.4 , -0.3]*	77	**0.005 (-0.2) *[-0.3 , -0.2]***	77	**0.001 (1.1) *[1.0 , 1.1]***

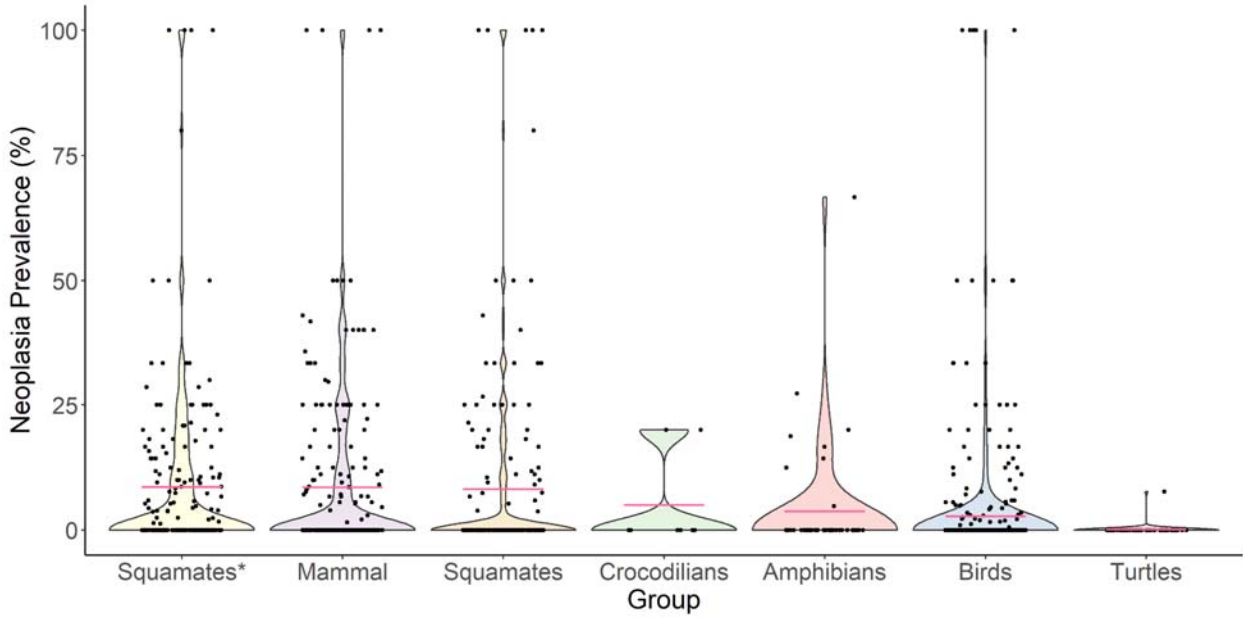
Vertebrate Group	Total Neoplasia				Cancer				Malignancy			
	Mass		Lifespan		Mass		Lifespan		Mass		Lifespan	
	N Species	p-value (slope) *[jack knife]*	N Species	p-value (slope) *[jack knife]*	N Species	p-value (slope) *[jack knife]*	N Species	p-value (slope) *[jack knife]*	N Species	p-value (slope) *[jack knife]*	N Species	p-value (slope) *[jack knife]*
Squamates	102	**0.02 (0.2)** ***[0.16 , 0.2]***	122	0.2 (0.4) *[0.3 , 0.6]*	102	0.14 (0.1) *[0.09 , 0.2]*	122	0.8 (0.08) *[-0.02 , 0.16]*	32	0.07 (-0.4) *[0.4 , -0.2]*	32	0.2 (-0.9) *[-1.1 , -0.7]*
Squamates*	116	**0.0003 (0.24)** ***[0.2 , 0.3]***	142	**0.0003 (0.9)** ***[0.8 , 1.1]***	116	**0.0004 (0.3)** ***[0.2 , 0.3]***	142	**0.0004 (1.07)** ***[1.0 , 1.2]***	56	0.6 (0.1) *[0.05 , 0.2]*	56	0.5 (0.4) *[0.07 , 0.6]*
Turtles	46	0.8 (0.07) *[0.05 , 0.07]*	42	0.8 (-0.3) *[-0.6 , 0.13]*	NA	NA	NA	NA	NA	NA	NA	NA

*Includes data from Duke et. al (2022)

FIGURES

Figure 1. Summary of (a) neoplasia prevalence and (b) cancer prevalence across tetrapod groups. Each point represents the prevalence of a single species. Pink lines represent the mean prevalence of each group. Groups ordered left to right by descending mean prevalence. Prevalence is based on data as in Table 1. *Indicates squamate data from Duke et al. (2022) included.

(a)



(b)

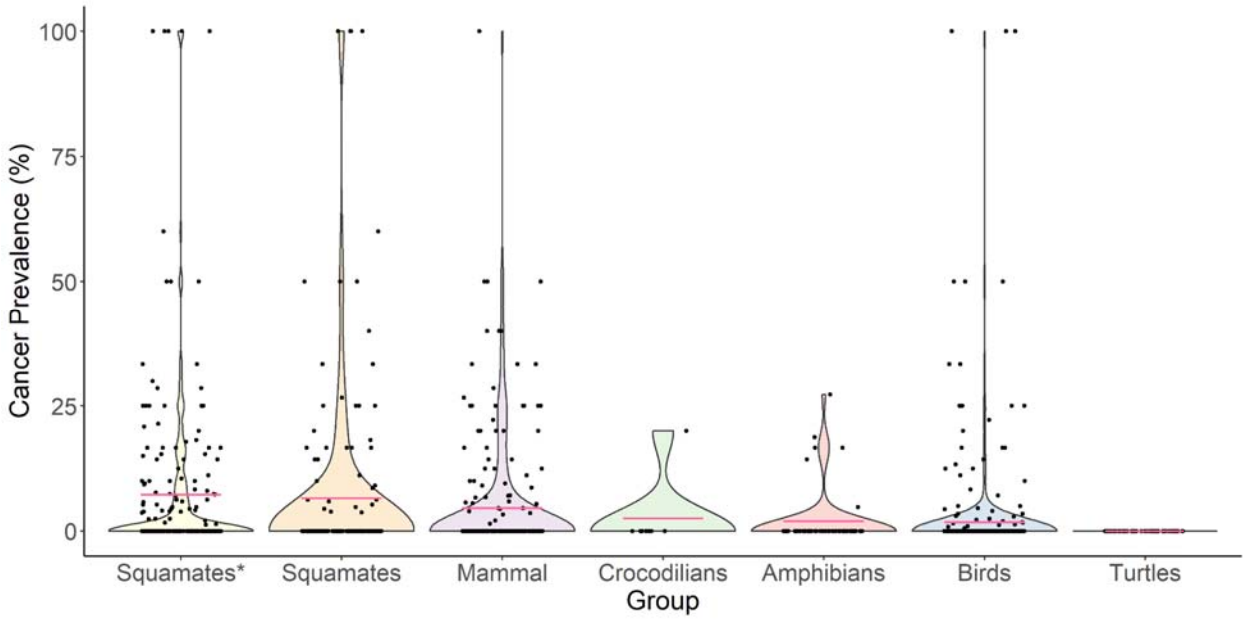

100
75
50
25
0
Cancer Prevalence (%)
Squamates*
Squamates
Mammal
Crocodilians
Amphibians
Birds
Turtles
Group

Figure 2. Prevalence of neoplasia, cancer, and malignancy across tetrapod groups using the full dataset as in Table 1. Points represent observed prevalence values and lines represent the 95% confidence intervals surrounding the observed prevalence value. *Indicates squamate dataset with data from Duke et al. (2022) included.

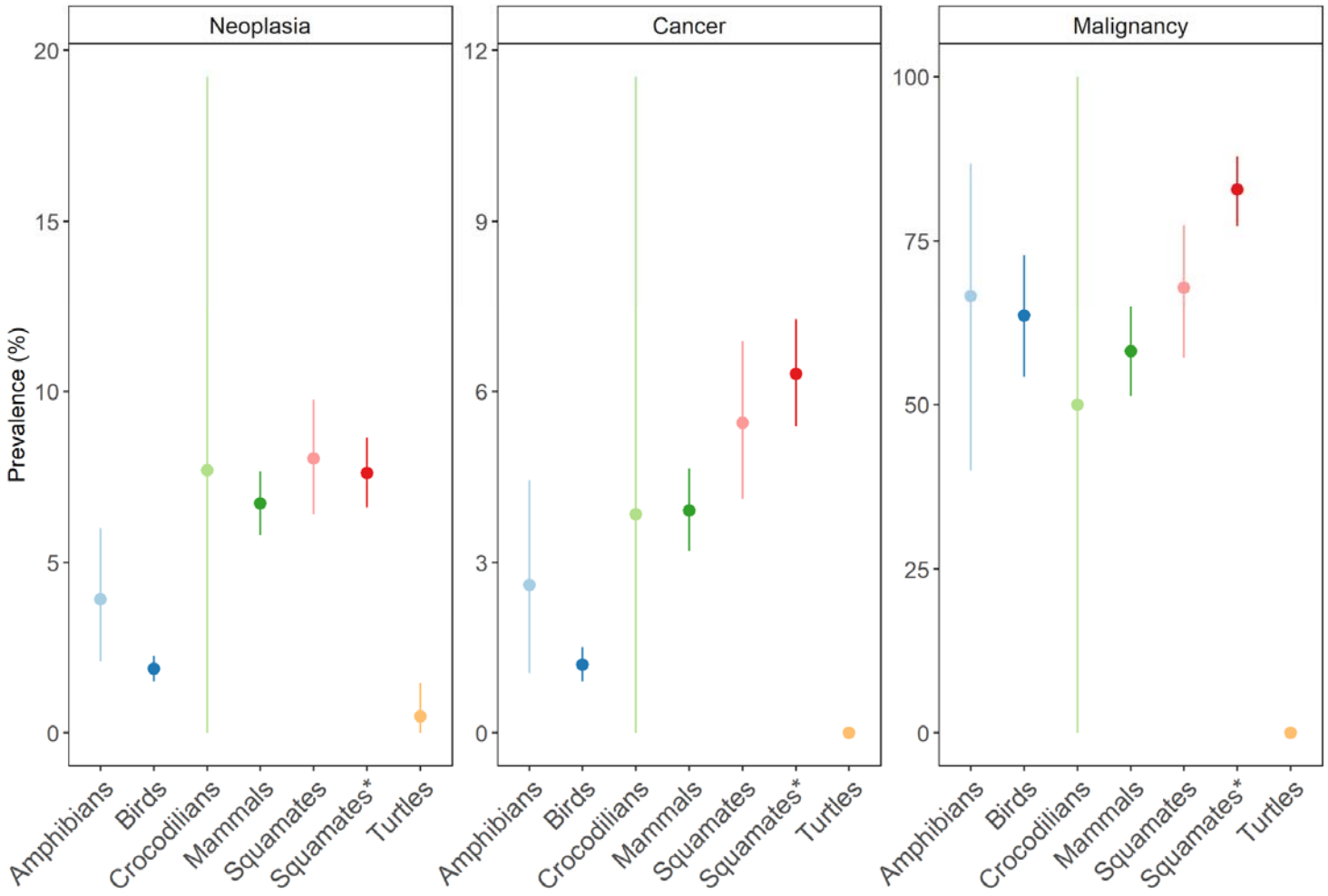
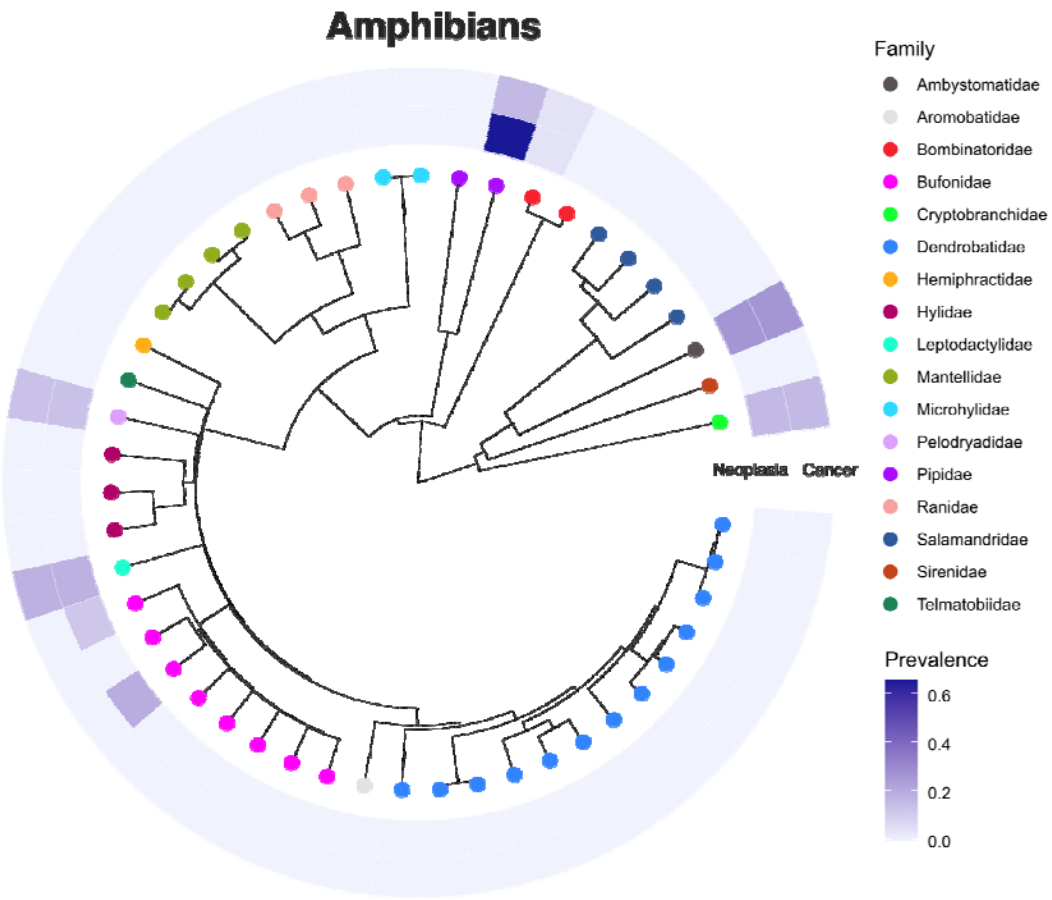
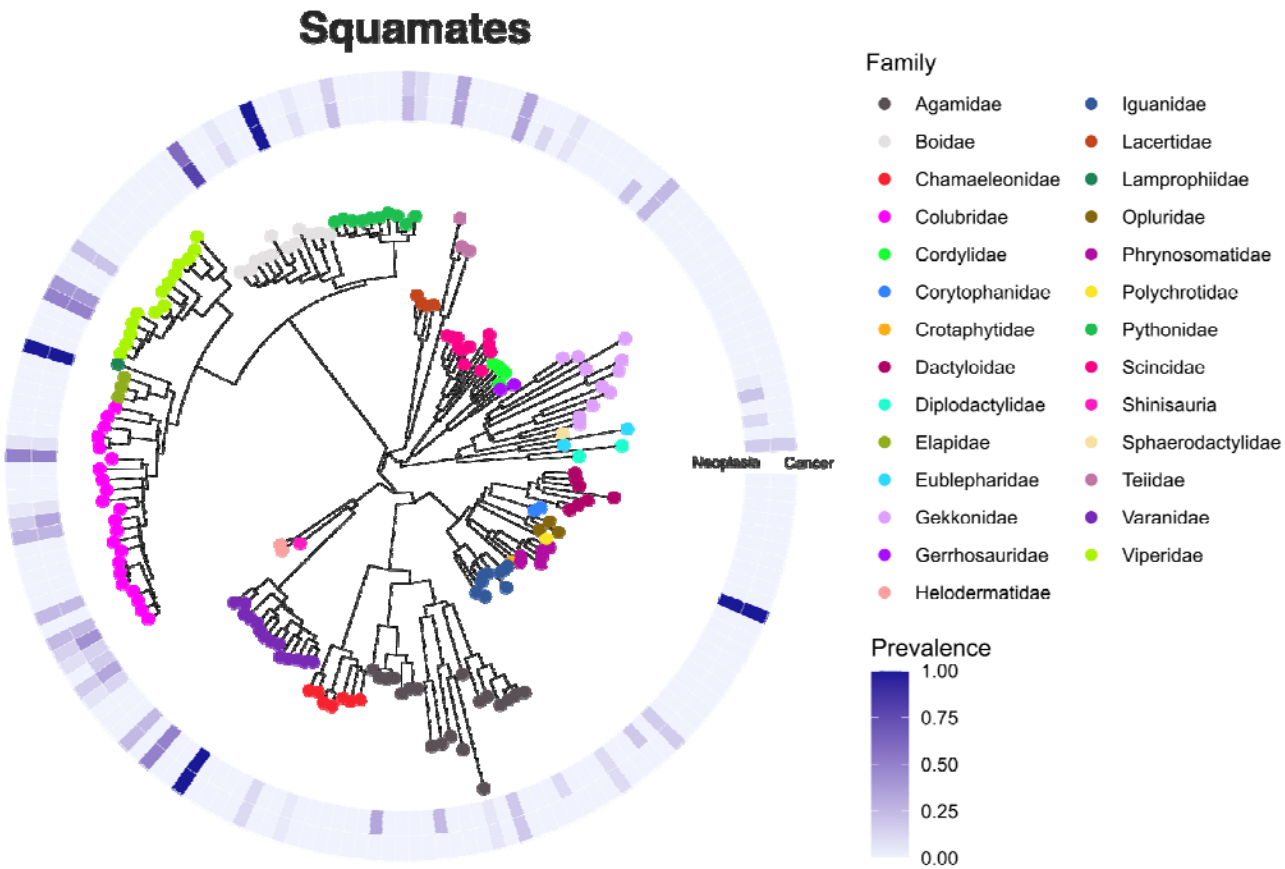


Figure 3. Interspecific variation in neoplasia and cancer prevalence within a) amphibian, b) squamates, c) mammals, and d) birds. Taxa are color coded either by family (amphibians and squamates) or order (mammals and birds).

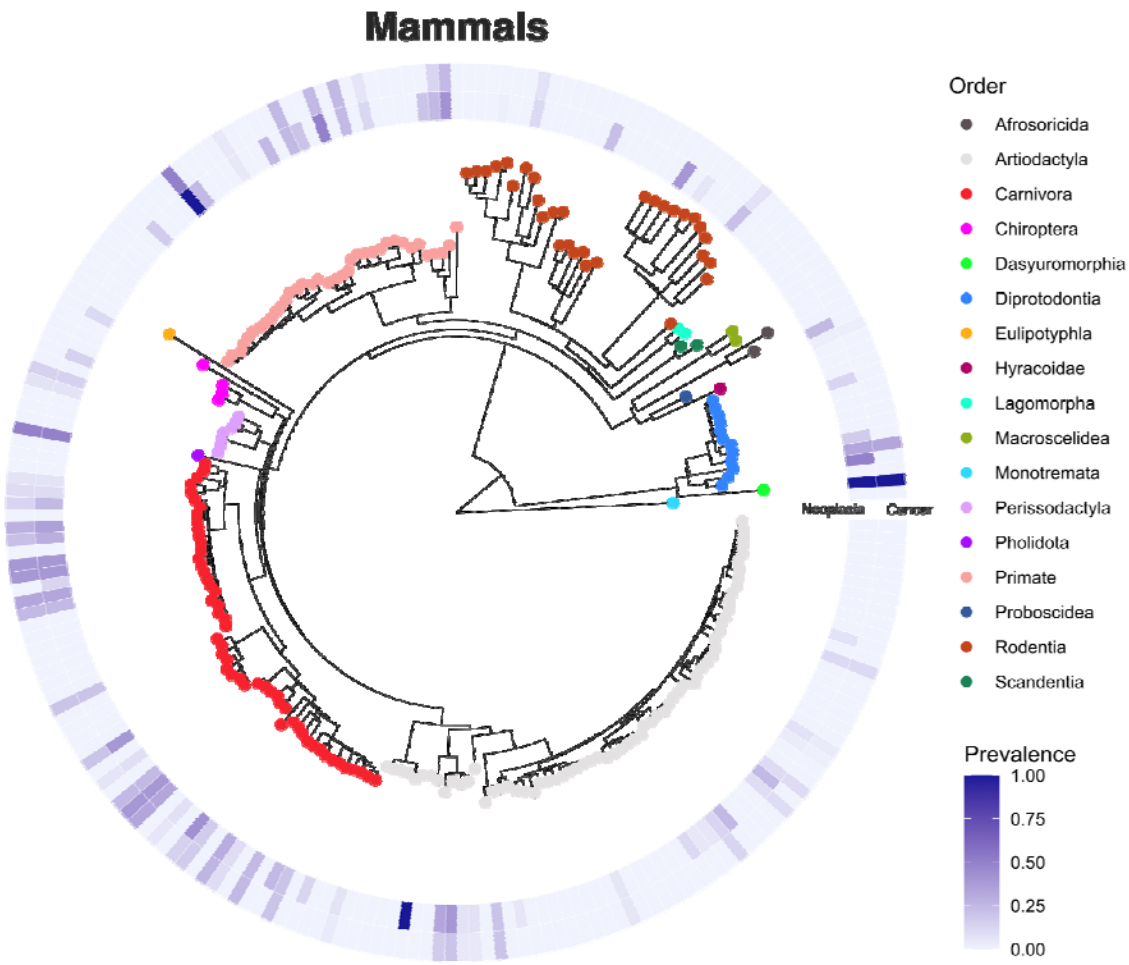
a)



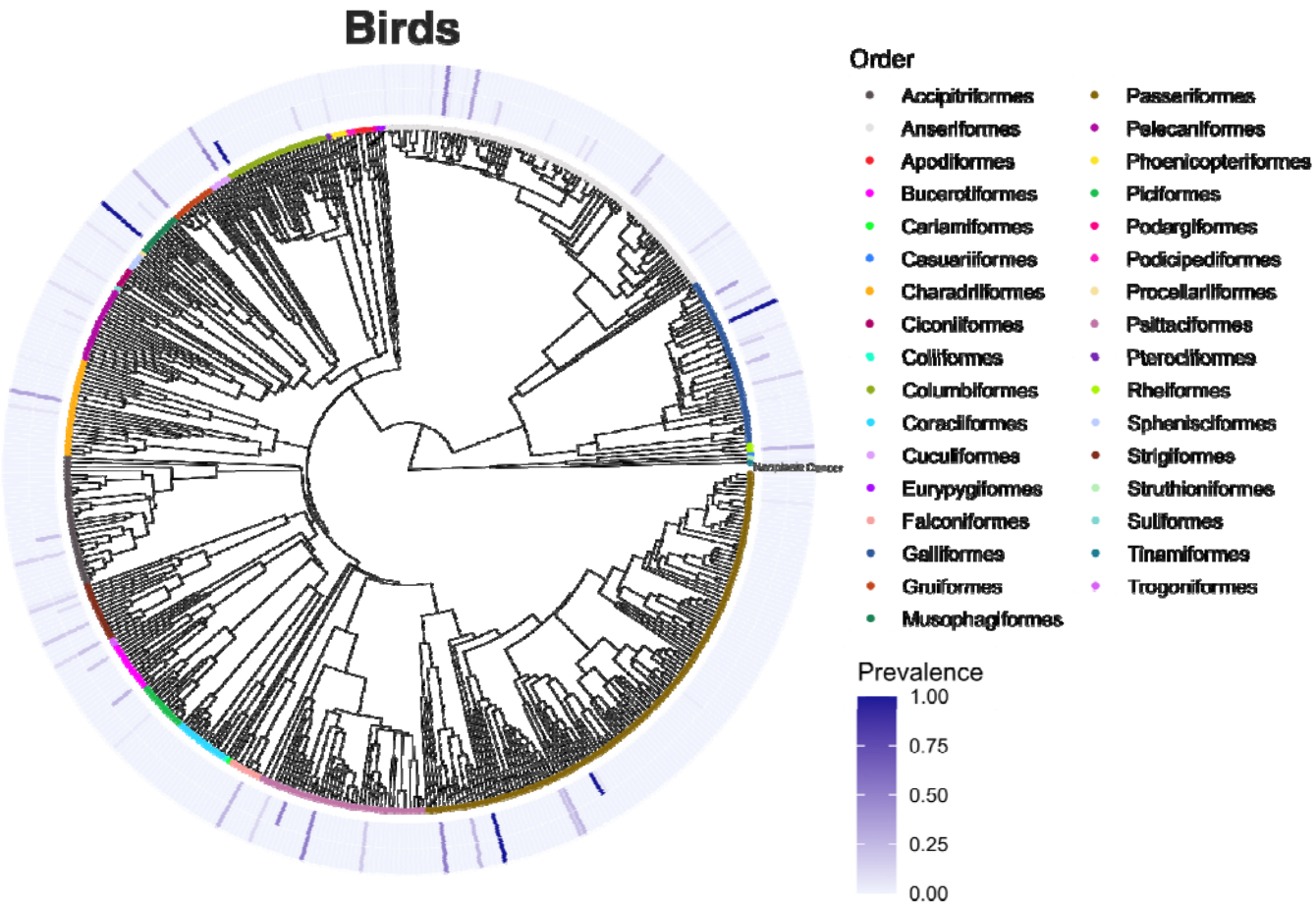
b)


Squamates
Neoplasia
Cancer
Family
Agamidae
Boidae
Chamaeleonidae
Colubridae
Cordylidae
Corytophanidae
Crotaphytidae
Dactyloidae
Diplodactylidae
Elapidae
Eublepharidae
Gekkonidae
Gerrhosauridae
Helodermatidae
Iguanidae
Lacertidae
Lamprophiidae
Opluridae
Phrynosomatidae
Polychrotidae
Pythonidae
Scincidae
Shinisauria
Sphaerodactylidae
Teiidae
Varanidae
Viperidae
Prevalence
1.00
0.75
0.50
0.25
0.00

c)


Mammals
Neoplasia
Cancer
Order
Afrosoricida
Artiodactyla
Carnivora
Chiroptera
Dasyuromorphia
Diprotodontia
Eulipotyphla
Hyracoidae
Lagomorpha
Macroscelidea
Monotremata
Perissodactyla
Pholidota
Primate
Proboscidea
Rodentia
Scandentia
Prevalence
1.00
0.75
0.50
0.25
0.00

d)


Birds
Order
Accipitriformes
Anseriformes
Apodiformes
Bucerotiformes
Cariamiformes
Casuariiformes
Charadriiformes
Ciconiiformes
Coliiformes
Columbiformes
Coraciiformes
Cuculiformes
Eurypygiformes
Falconiformes
Galliformes
Gruiformes
Musophagiformes
Passeriformes
Pelecaniformes
Phoenicopteriformes
Piciformes
Podargiformes
Podicipediformes
Procellariiformes
Psittaciformes
Pteroclidiformes
Rheiformes
Sphenisciformes
Strigiformes
Struthioniformes
Suliformes
Tinamiformes
Trogoniformes
Prevalence
1.00
0.75
0.50
0.25
0.00

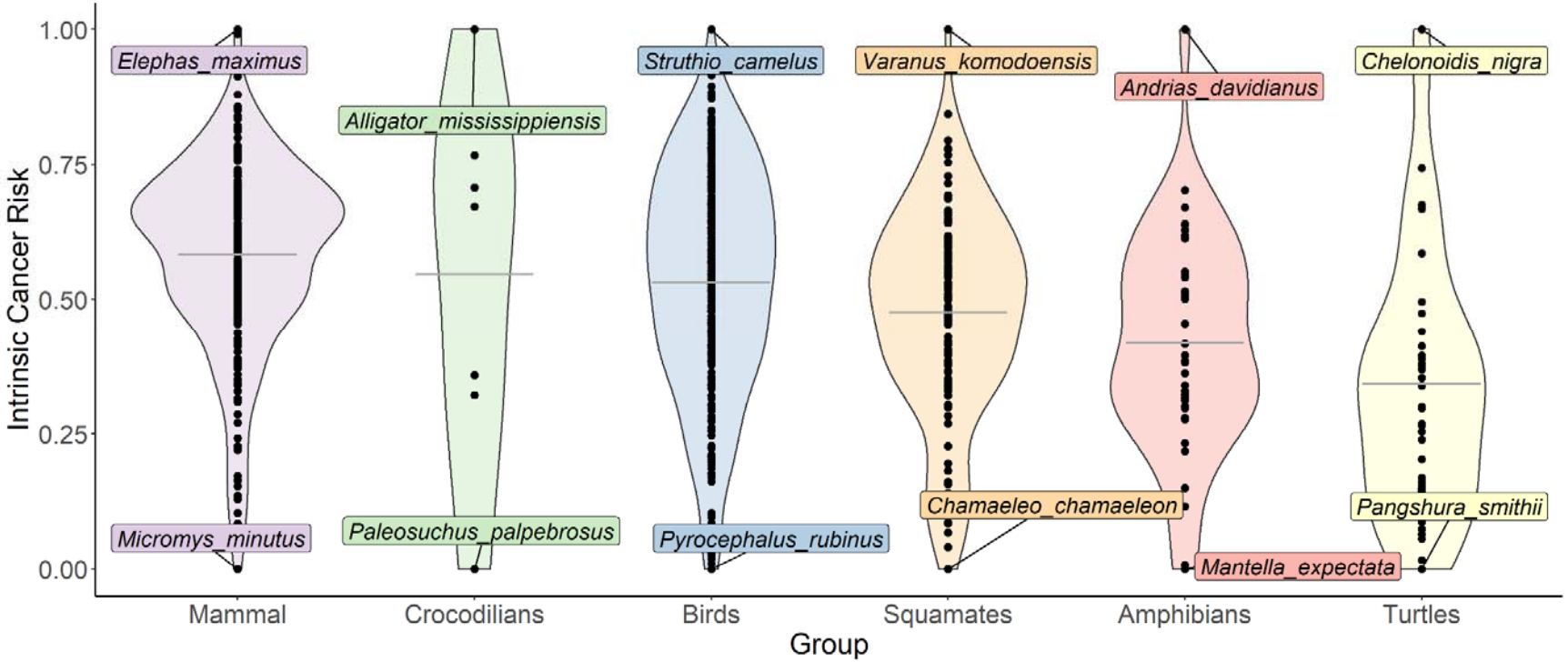



Figure 4. Estimated intrinsic cancer rates (ICR) based on body mass and lifespan data for all tetrapod species examined in this study (see Materials and Methods for ICR calculation). Gray lines in each violin represent the mean ICR for the tetrapod group.