A real data-driven simulation strategy to select an imputation method for mixed-type trait

data

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Abstract

Missing observations in trait datasets pose an obstacle for analyses in myriad biological

disciplines. Considering the mixed results of imputation, the wide variety of available methods,

and the varied structure of real trait datasets, a framework for selecting a suitable imputation

method is advantageous. We invoked a real data-driven simulation strategy to select an

imputation method for a given mixed-type (categorical, count, continuous) target dataset.

Candidate methods included mean/mode imputation, *k*-nearest neighbour, random forests, and

multivariate imputation by chained equations (MICE). Using a trait dataset of squamates (lizards

and amphisbaenians; order: Squamata) as a target dataset, a complete-case dataset consisting of

species with nearly complete information was formed for the imputation method selection.

Missing data were induced by removing values from this dataset under different missingness

mechanisms: missing completely at random (MCAR), missing at random (MAR), and missing

not at random (MNAR). For each method, combinations with and without phylogenetic

information from single gene (nuclear and mitochondrial) or multigene trees were used to impute

the missing values for five numerical and two categorical traits. The performances of the

methods were evaluated under each missing mechanism by determining the mean squared error

and proportion falsely classified rates for numerical and categorical traits, respectively. A

random forest method supplemented with a nuclear-derived phylogeny resulted in the lowest

error rates for the majority of traits, and this method was used to impute missing values in the

original dataset. Data with imputed values better reflected the characteristics and distributions of

the original data compared to complete-case data. However, caution should be taken when

imputing trait data as phylogeny did not always improve performance for every trait and in every

scenario. Ultimately, these results support the use of a real data-driven simulation strategy for

selecting a suitable imputation method for a given mixed-type trait dataset.

Author summary

The issue of missing data is problematic in trait datasets as the missingness pattern may not be

entirely random. Whether data are missing may depend on other known observations in the

dataset, or on the value of the missing data points themselves. When only complete cases are

used in an analysis, derived results may be biased. Imputation is an alternative to complete-case

analysis and entails filling in the missing values using information provided by other trait values

present in the dataset. Including phylogenetic information in the imputation process can improve

the accuracy of imputed values, though results are dependent on the amount and pattern of

missingness. Most previous evaluations of imputation methods for trait datasets are limited to

numerical simulated data, with categorical traits not considered. Given a particular dataset, we

propose the use of a real data-driven simulation strategy to select an imputation method. We

evaluated the accuracies of four different imputation methods, with and without phylogeny

information, and under different simulated missingness patterns using an example reptile trait

dataset. Results indicated that data imputed using the best-performing method better reflected the

original dataset characteristics compared to complete-case data. As imputation performance

varies depending on the properties of a given dataset, a real data-driven simulation strategy can

be used to provide guidance on best imputation practices.

Introduction
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Trait data are used in a wide variety of biological disciplines, including evolutionary

biology, community ecology, and biodiversity conservation. For instance, trait data pertaining to

the life history of a species, such as longevity, metabolic rate, and generation time, are integral in

studies of biological aging [1,2]. Environmental trait data, such as latitude, temperature, and

habitat type, may be used to identify those species most at risk of extinction [3,4]. However, an

extensive proportion of these trait data are often missing. Missingness may stem from a

taxonomic bias: data are available in copious amounts for well-researched or charismatic species

and are lacking for endangered species or those that inhabit remote environments (e.g. deep sea)

[5–7]. Mammal and bird taxa tend to be well sampled, and data for a large and diverse array of

traits are available for many groups [8,9]. However, regional and phylogenetic biases are

common in trait data for groups such as reptiles and amphibians, and observations are largely

limited to body size and habitat traits [9]. Species traits are often tied to evolutionary history, a

concept referred to as phylogenetic signal [10]. Closely related species can share the

characteristics that render them elusive or difficult to study (e.g. small body size), resulting in

patchy or unreliable data for entire taxonomic clades [5,6,8]. Certain types of trait data may also

be easier to quantify (e.g. morphometric data) as opposed to traits that require arduous or

invasive data collection techniques (e.g. age or reproductive data) [11] (see Fig 1 for a

visualization of missingness in reptiles). When trait datasets are used in biological studies, these

biases can lead researchers to make erroneous conclusions about the data. Consequently, the

development of approaches and guidelines for handling missing data is an important area of

research that spans across multiple biological disciplines.

Fig 1. Visualization of missingness. Visualization of missingness (proportion of present vs.

missing observations) in Squamata trait data obtained from the primary literature. Superscripts

indicate the original sources of the trait data: 1) amniote life history database [12,13], 2)

vertebrate home range sizes dataset [14,15], 3) traits of lizards of the world [16,17] and 4)

AnAge [18,19]. See S1 File for further detail on trait sources.

The use of complete-case datasets can result in a large proportion of information being

discarded [7,20]. More specifically, a loss in statistical power can result upon the removal of

many incomplete cases if the data are "missing completely at random" (MCAR) [7,21]. Trait

data, however, are often "missing at random" (MAR): observations that are missing for a

particular trait are related to known values for other traits in the dataset. Simply removing

incomplete cases when data are MAR can result in a reduction in statistical power and biased

estimations of model parameters [7,11,22]. In more extreme cases, trait data may be "missing not

at random" (MNAR): the reason data are missing is related to the unobserved data themselves. In

such scenarios, the reason for missingness may be unclear to the researcher and thus difficult to

verify empirically [23].

Imputing missing observations is a common alternative to the complete-case analysis.

Imputation techniques use known observations to estimate the missing and unobserved values of

a variable (or variables) of interest. Single imputation techniques such as hot deck imputation or

k-nearest neighbour (KNN) [24] offer an efficient means for estimating missing values; however,

these methods provide only a single estimate of the missing value. Random forest methods such

as missForest [25] are also growing in popularity as they make no prior assumptions about the

distributions of variables. Multiple imputation techniques have been developed that perform

single imputation several times and are therefore capable of providing a measure of uncertainty

of the imputed values [7,26]. An example of a multiple imputation method is multivariate

imputation by chained equations (MICE; 27], which offers numerous models for imputing data

of different types. Incorporating phylogenetic information into the imputation process has also

been shown to increase the accuracy of imputed values [11,28]. This increase in accuracy is a

result of the phylogenetic signal that is often inherent in trait data. A commonly used method for

incorporating phylogenetic information into the imputation process is the use of phylogenetic

eigenvectors. More specifically, methods such as phylogenetic eigenvector regression (PVR)

[29] and phylogenetic eigenvector mapping (PEM) [30] employ a principal coordinates analysis

(PCoA) to derive eigenvectors from a phylogenetic tree. PEM expands on the PVR method by

applying an additional branch length transformation based on the Ornstein-Uhlenbeck

evolutionary model [30,31]. Phylogenetic eigenvectors may then be used as additional predictor

variables in the imputation process [11,32,33].

As missing data are a major concern in trait datasets, we are motivated to consider

imputing these values. The correlative nature of trait data makes them suitable candidates for

imputation, particularly when phylogenetic signal is also present [34]. When phylogenetic signal

is strong for a trait, as may be the case for recently diverged groups, species may be more likely

to exhibit similar values of the trait; these relationships can then be utilized by imputation

methods to improve estimates. In an evaluation of imputation methods using mammalian trait

data, Penone *et al.* [11] found that supplementing the imputation process with phylogenetic

information improved the accuracy of KNN, missForest, and MICE for several life history traits.

Kim *et al.* [32] similarly found that adding phylogenetic information to MICE improved

accuracy rates of estimated functional diversity metrics. However, when imputing bird

demographic traits with moderate phylogenetic signal (Pagel's $\lambda < 0.8$), James *et al.* [35] found

that use of phylogenetic information improved error rates by a margin of less than 1%.

Moreover, they suggest that the use of auxiliary traits (traits that are present in the dataset but not

the target of imputation) were often sufficient for accurate imputations. In sum, these findings

indicate that improvements conferred by phylogenetic imputation methods are context-

dependent, contingent upon the presence of phylogenetic signal and relationships among traits in

the dataset.

Trait data exist in several forms, ranging from the discrete categories of foraging

behaviour to the countable number of eggs in a nest. Many contemporary imputation methods

are able to estimate mixed-type data, including both categorical and numerical (continuous and

count) values. However, several previous studies have only evaluated their performances using

simulated trait data [32,34], and the studies that have utilized real data are limited to numerical

traits [11,36]. Given the diversity of available trait datasets and the varied success of imputation

methods in comparative evaluations using trait data [11,34], additional guidelines are needed

regarding imputation method selection. Identifying an imputation method that is most suitable

for a given dataset, including its combinations of variables and biological properties, would be

advantageous in reducing error rates when imputing missing trait values. Here, we propose a real

data-driven strategy to select an imputation method for a given target trait dataset. The strategy

entails four main steps: 1) missingness simulations under MCAR, MAR, and MNAR

mechanisms, 2) imputation of simulated missing values using different candidate methods, 3)

identification of the best-suited imputation method based on their performances, and 4)

application of the best-suited imputation method to the target dataset and comparison between

original, imputed, and complete-case data characteristics. The best-suited imputation method for

the dataset is defined as the method, with or without phylogeny, that results in the lowest

imputation error rates overall for both numerical and categorical traits. The real data simulation

results can provide additional insights about the dataset in question including 1) whether there is

an imputation method that performs best (i.e. results in the lowest imputation error rates) for a

specific data type (continuous, count, categorical) and/or missingness mechanism (MCAR,

MAR, and MNAR); 2) whether phylogenetic information improves the imputation performance;

and 3) which type of phylogenetic information is influential (mitochondrial, nuclear).

Phylogenetic resolution varies among gene trees [37,38], and certain genes may be more or less

suited for imputation in a given taxon and taxonomic rank. Together, these insights can help

determine a target dataset's suitability for imputation including whether phylogenetic imputation

is useful and if some traits are more amenable to imputation than others. The method-selection

strategy proposed here may be considered for future trait-based analyses to avert biases

associated with the use of complete case and to mitigate error rates by employing a well-suited

imputation method. In turn, this can facilitate the pursuit of new research directions, particularly

in those fields impeded by patchy and incomplete trait data.

Results

Performance comparison without phylogeny

Imputation performance was measured using mean squared error (MSE) and proportion

falsely classified (PFC) for numerical and categorical traits, respectively. In both cases, lower

error rates are indicative of better performance. In general, when missing data were simulated

under MCAR, error rate increased with missingness proportion; this trend was observed for all

trait and method combinations (Fig 2). Under the same simulation settings, *k*-nearest neighbour

(*KNN)* [24,39], random forests (*RF*; "missForest" R package) [25,40] and multivariate

imputation by chained equations (*MICE)* [27] outperformed mode and mean imputation for the

majority of traits. However, for the multi-level categorical trait activity time, mode imputation

resulted in a lower error rate than *RF* and *KNN* at 30-40% missingness (Fig 2a). Additionally, for

smallest clutch, the mean imputation method outperformed *KNN* (10-40%) and *RF* (10%, 30-

40%) (Fig 2d). *MICE* resulted in lower error rates than *KNN* and *RF* for most traits across all

missingness proportions. However, *KNN* resulted in the lowest error rate for activity time at 10%

missingness, and *RF* resulted in the lowest error rate across all missingness proportion settings

for the insular endemic trait (Fig 2b). Under MAR without phylogeny, *MICE* generally

outperformed both *RF* and *KNN* (see Figs 3 and 4); however, results were mixed for MNAR

scenarios without phylogeny as the prevailing method depended on the trait in question. For

example, *KNN* performed best for smallest clutch under MNAR without phylogeny (Fig 4b)

whereas *RF* performed best for both categorical traits (Fig 3).

Fig 2. MCAR performance without phylogeny. Performances of the methods mean/mode

imputation, *KNN*, missForest (*RF*), and multivariate imputation by chained equations (*MICE*)

across different proportions of missingness when data were missing completely at random

(MCAR). For MICE, logistic regression and predictive mean matching were used for imputing

categorical and numerical traits, respectively. Error rate was measured as proportion falsely

classified (PFC) for the categorical traits a) activity time and b) insular endemic and as mean

squared error (MSE) for the numerical traits c) largest clutch, d) smallest clutch, e) latitude, f)

female snout-vent length (SVL), and g) maximum SVL. PFC and MSE values for each trait were

averaged across 100 dataset replicates. In both cases, error rates closer to 0 are indicative of

better performance.

Fig 3. Imputation performance under different missingness mechanisms for categorical

traits. Comparison of error rates (PFC) for the methods mean/mode imputation, *KNN*, *RF*, and

MICE under different missingness mechanisms with and without the addition of phylogenetic

information. Phylogenetic information was added in the form of trees built from sequence data of

mitochondrial cytochrome *c* oxidase subunit I (COI), nuclear oocyte maturation factor (c-mos),

recombination activating gene 1 (RAG1), and a composite multigene (MG) tree. Performances

were quantified using PFC for the categorical traits a) activity time and b) insular endemic. PFC

values for each trait were averaged across 100 dataset replicates. MAR = missing at random;

MNAR = missing not at random.

Fig 4. Imputation performance under different missingness mechanisms for numerical

traits. Comparison of error rates for the methods mean/mode imputation, *KNN*, *RF*, and *MICE*

under different missingness mechanisms with and without the addition of phylogenetic

information. Phylogenetic information was added in the form of trees built from sequence data of

COI, c-mos, RAG1, and a composite multigene (MG) tree. Performances were quantified using

MSE for the numerical traits a) largest clutch, b) smallest clutch, c) latitude, f) female SVL, and

g) maximum SVL. MSE values for each trait were averaged across 100 dataset replicates.

Phylogenetic imputation performance

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All traits exhibited significant phylogenetic signal in all gene trees (S1 Fig; see S1 File

for more details on phylogenetic signal measures), and phylogenetic information was

incorporated into the imputation process using phylogenetic eigenvectors (see SI). Although

adding phylogeny was generally beneficial, improvements to imputation performance were

contingent on method, data type, and missingness mechanism (Figs 3 and 4). For instance, when

considering the categorical traits activity time and insular endemic, supplementing phylogenetic

information from any of the trees generally improved performance for each method and under

each missingness mechanism (Fig 3). Under the MAR scenario for activity time, however,

adding RAG1 information to *MICE* resulted in an increased error rate relative to the scenario

without phylogeny (Fig 3a). For most traits, MAR results reflected those under the MCAR

mechanism; however, deviations from the general pattern occurred in some MNAR cases. For

example, under MNAR for female SVL and maximum SVL, adding information from any tree

increased error rates for both *KNN* and *RF* (Fig 4d,e).

For the traits largest clutch, smallest clutch, and latitude, *KNN*, *RF,* and *MICE*

performances were improved (or remained neutral) after the addition of any type of phylogenetic

information under MCAR, MAR, and MNAR (Fig 4a-c). However, which tree resulted in the

best imputation performance varied depending on the trait, method, and missingness mechanism.

For example, *RF* with c-mos performed best for largest clutch under MCAR 40% (Fig 4a),

whereas *KNN* with COI or multigene ("MG") performed best for smallest clutch under MNAR

(Fig 4b). The traits female snout-vent length (SVL) and maximum SVL displayed somewhat

dissimilar patterns from the other traits (Figs 4d,e) as phylogenetic information decreased

imputation performance or had no substantial effect in most scenarios.

The relationship between phylogenetic signal and error ratio (error rate without

phylogeny/error rate with phylogeny) varied depending on data type. For categorical traits,

higher error ratio, indicative of better performance due to phylogeny, was generally associated

with higher phylogenetic signal strength (Fig 5a). This same pattern was not as apparent for

numerical traits (Fig 5b). Moreover, under the MNAR mechanism for numerical traits, several

error ratio values fell below 1 with stronger phylogenetic signal, indicative of a reduction in

performance due to phylogeny.

Fig 5. Association between error ratio and phylogenetic signal. Association between error

ratio (error rate without phylogeny/error rate with phylogeny) and phylogenetic signal (Fritz and

Purvis' *D* [41] for categorical traits and Pagel's λ [42] for numerical traits) at different

proportions of missingness. Error ratio values above 1 (indicated by the gray line) signify an

improvement in performance when phylogeny is added. In the case of *D,* lower values are

indicative of higher levels of phylogenetic conservation for the trait; conversely, higher values of

λ suggest stronger phylogenetic signal. Results are not shown for MAR in a) as only one trait

(activity time) was simulated under this mechanism. To improve visualization, values were

jittered (random noise introduced to data) using the package "ggplot2" [43]. Additionally, results

are only shown for the c-mos gene (categorical traits) and RAG1 gene (numerical traits) as other

genes followed similar patterns and these genes displayed the greatest range in phylogenetic

signal.

Imputation of original dataset using best-suited method

258   Using our majority voting system, the method that resulted in the lowest error rates for

the majority of traits was *RF* with c-mos. Consequently, *RF* with c-mos was considered as the

best-suited method for this dataset and used to impute the missing values in the target trait

dataset (hereafter, "original dataset"). Out of the total species in the original dataset ($n = 6657$),

those with available information for any of the seven traits as well as available c-mos sequence

records were included in the subset of original data to be imputed ($n = 921$). The proportion of

missingness varied for each trait in this original data subset as 0.16 for activity time, 0 for insular

endemic, 0.21 for largest clutch, 0.21 for smallest clutch, 0.23 for female SVL, 0 for maximum

SVL, and 0 for latitude. As insular endemic, maximum SVL, and latitude had complete

observations in this subset, these traits did not require imputation.

Distributions and categorical frequencies of the complete-case, original, and original data

with imputed values (hereafter, "imputed data") can be observed in Fig 6. For the trait activity

time, when compared to the original data, discrepancies in the categorical frequencies were more

apparent in the complete-case data than in the imputed data (Fig 6a). The complete-case data

displayed a greater overrepresentation of the rarest category (cathemeral: 11% vs. 8.9%) and

underrepresentation of the most common category (diurnal: 57.9% vs. 64.4%). Conversely, the

imputed data displayed a greater representation of observations in the most common category

compared to the original data (diurnal: 67.5% vs. 64.4%). For all numerical traits, the imputed

data distributions followed the distributions of the original more closely than did the complete-

case distributions (Fig 6b-d; Table 1). Perhaps most apparent are the discrepancies in the

maximum values in the complete-case data compared to those in the original and imputed data

(e.g. for largest clutch, 68 vs. 88; for smallest clutch, 8 vs. 30). Although both complete-case and

imputed data displayed reduced variance relative to the original data for the traits largest clutch,

smallest clutch, and female SVL, the loss in variance for the complete-case data was

considerably greater for all traits.

Fig 6. Comparison of quantitative characteristics across datasets. Comparison of a)

categorical frequencies for the trait activity time and distributions for the traits b) largest clutch,

c) smallest clutch, and d) female SVL of the complete-case data, original data, and imputed data.

The natural logarithm (ln) of the numerical data were taken to improve visualization.

Table 1. Summary statistics for the complete-case data, original data, and original data with imputed values.

	Largest clutch (# eggs/neonates)			Smallest clutch (# eggs/neonates)			Female SVL (mm)			Maximum SVL (mm)		Latitude (°)	
	CC	*O*	*I*	*CC*	*O*	*I*	*CC*	*O*	*I*	*CC*	*O*	*CC*	*O*
N	141	731	921	141	731	921	137	705	921	152	921	152	921
Min	1	1	1	1	1	1	18.7	18.7	18.7	21.7	21.7	-40.36	-47.89
Max	68	88	88	8	30	30	499.5	534.3	534.3	1170	1170	56.6	56.6
Range	67	87	87	7	29	29	480.8	515.6	515.6	1148.3	1148.3	96.96	104.49
Median	2	3	3	1	2	2	60.1	62.7	65.2	77	80	-11.36	-9.48
Mean	5.79	6.08	6.06	1.63	2.06	2.14	75.82	83.4	84.36	103.44	110.35	1.65	-3.8
SE (mean)	0.67	0.33	0.27	0.09	0.07	0.06	4.76	2.43	2.07	8.58	3.30	2.03	0.75
0.95 CI (mean)	1.33	0.65	0.53	0.18	0.14	0.12	9.41	4.76	4.06	16.96	6.48	4.01	1.48
Variance	63.85	79.46	67.41	1.22	3.90	3.46	3103.49	4151.01	3950.16	11197.05	10048.39	627.38	521.53
Standard deviation	7.99	8.91	8.21	1.10	1.98	1.86	55.71	64.43	62.85	105.82	100.24	25.05	22.84

Summary statistics of the complete-case (CC), original (O), and original data with imputed values (I) for the numerical traits largest clutch, smallest clutch, female snout-vent length (SVL), and latitude. As the proportion of missingness was 0 for the traits maximum SVL and latitude in the original data subset, these traits were not imputed. Original trait data obtained from Meiri [16,17].

Discussion

To select a best-suited imputation method for the given squamate trait dataset, performances for four candidate methods were evaluated in various scenarios: under different missingness mechanisms and both with and without phylogeny. In alignment with previous evaluations of imputation methods using trait data [11,34,44], the method that performed best (resulted in the lowest error rates) varied in accordance with the simulation settings. When the candidate methods are considered without phylogeny (Fig. 2), as may be the case when molecular data for a taxon are unavailable or of low quality, the best overall method was *MICE*. *MICE* demonstrated strong performances in previous evaluations of imputation techniques in mammalian [11] and plant [44] trait datasets. The robustness of the predictive mean matching model is appealing for the non-linear relationships and non-normal distributions commonly observed in numerical trait data [45,46]. This may explain the superior performance of *MICE* in the case of smallest clutch, a count trait with a right-skewed distribution (many species with smallest clutch size = 1). Predictive mean matching has also been shown to perform well on smaller sample sizes [46], as seen in the current study ($n = 152$). Its use in trait imputation is therefore an appealing option when sequence data are scarce or phylogenetic signal in the data is weak.

As reported in previous studies [11], imputation error rates tended to increase with missingness proportion and varied amongst different traits. Indeed, for some traits at high missingness percentages, methods performed similarly or worse than mode/mean imputation. These poor performances may be explained by the distributional properties of the trait in question; for example, when considering the trait activity time (see Fig. 2a), a loss of information

in this multi-categorical trait due to large proportions of missing data (30-40%) reduced the

imputation accuracy for *RF*. This is an issue that can be mitigated through the addition of

phylogeny, as imputation accuracy was improved for activity time at high missing proportions

when information from any tree was added (see Fig. 3a). However, adding phylogenetic

information does not always improve imputation performance; on the contrary, in some instances

its inclusion led to increased error rates. The effect of phylogeny therefore appears to be

situational and linked to the method used, the underlying mechanism of the missingness in the

data, and quantitative attributes and evolutionary history of the target trait.

As phylogenetic resolution varies between nuclear and mitochondrial gene trees, the

number of eigenvectors used for imputation varied in accordance. In this study, the threshold

method was used to determine the number of eigenvectors to be included; however, it is possible

that the use of too many eigenvectors (i.e. > 40) [47], with less information provided by each

eigenvector, can introduce more noise or lead to overfitting. *MICE* was particularly sensitive to

changes in the number of eigenvectors, as the inclusion of many (> 20) tended to drastically

increase error rates. A similar increase in error rate was found in Johnson *et al.* [34] when

phylogenetic information was added to *MICE*, especially under MNAR (e.g. larger values more

likely to be missing). Penone *et al.* [11] restricted their maximum number of eigenvectors to 10

and suggest that the use of too many eigenvectors can mask the information provided by other

traits in the imputation process. Indeed, in the current study, *MICE* performed well when the

number of predictors were low, as in the case of trait-only imputation and when the number of

eigenvectors were limited. Thus, analyses using phylogenetic eigenvectors for imputation may

consider the use of tree-based methods such as *RF* (or recursive partitioning; see Kim *et al.* [32])

that are more robust to high-dimensional data.

335   The best-suited method overall for the given dataset was identified as *RF* with c-mos.

336 This method resulted in the lowest error rates for the majority of traits, when all missingness

337 mechanisms were considered. This result supports previous evaluations of the effectiveness of

338 *RF* for mixed-type data [25]. For all methods, adding phylogenetic information reduced

339 imputation error rate for traits of all types (binary, categorical, count, continuous); however, the

340 amount of error reduction varied according to the method and tree used. For example, trees built

341 using nuclear-derived phylogenetic information (i.e. c-mos, RAG1, MG) generally conferred a

342 greater improvement in imputation performance relative to mitochondrial COI for the trait

343 insular endemic; on the contrary, COI information was more useful for the trait smallest clutch.

344 Due to their faster rates of nucleotide substitution, mitochondrial genes are less adept at

345 resolving deeper phylogenetic relationships relative to nuclear genes [48]. However, COI often

346 still conferred a reduction in imputation error rate, in some cases more so than the nuclear genes;

347 mitochondrial sequences therefore should be used when nuclear data are unavailable and may be

348 advantageous when studying more closely related species. Overall, these results demonstrate the

349 applicability of single gene trees for imputation when high-quality sequence data are scarce, as

350 may be the case for understudied taxa.

351   Strength of phylogenetic signal also appeared to correlate with error ratio (i.e. the

352 magnitude of performance enhancement) for categorical traits. The same pattern was not as

353 apparent for numerical traits, however. This may stem from the limited range of phylogenetic

354 signal observed for these other types: all genes displayed significant levels of phylogenetic signal

355 for all traits, many of which verged toward $\lambda = 1$ (higher trait conservation). This may suggest

356 that the boost in performance due to phylogeny is negligible beyond a certain level of

phylogenetic signal. However, imputation of a greater number and variety of traits that do not

display any evidence of phylogenetic signal would need to be included to test this assertion.

The comparison between the distributions and categorical frequencies of the complete-

case, original, and imputed trait data support the efficacy of imputation for mixed-type data when

the method is carefully chosen. A greater than 6-fold increase in sample size when using imputed

data ($n = 152$ for complete-case vs. $n = 921$ for imputed data) is striking and illustrates the

information loss that can occur when using a complete-case approach. Complete-case data often

do not capture the true variability of the data; instead, they comprise a biased subset and, in turn,

the potential for erroneous inferences. Previous studies using clinical data [49] and mammalian

trait data [11] found that inferences derived from imputed datasets are less biased when

compared to those obtained using complete-case datasets. However, the missing values in these

studies were introduced either completely at random (MCAR) or at random (MAR). Although

imputation performs well under MCAR and MAR, the mechanism of missingness is often

difficult to determine in practice [23,50]. Imputation has been shown to perform poorly in

scenarios with biased missingness, such as when extreme values or values in the tails of the

distribution of the population are disproportionately missing [34]. If data are truly MNAR and

the imputation method is not carefully chosen, imputed values and the inferences derived therein

may be inaccurate. A recent study completed by Jardim *et al.* [51] suggests that accurate

estimation of phylogenetic signal from imputed datasets is contingent on several variables,

including the amount of missing data, missing mechanism, and the evolutionary trajectory of the

trait itself. For example, in the current study, as values closer to the equator were missing for the

latitude trait under MNAR, mean imputation outperformed most other imputation methods. Due

to the prevalence of allopatric speciation modes in diversification [52,53], closely related species

can inhabit different latitudes or distributions; traits with such evolutionary histories may be less

suitable for imputation. Therefore, we agree with Johnson *et al.* [34] and Jardim *et al.* [51] that

caution should be taken and the properties of the dataset of interest be inspected prior to and

following imputation. Testing imputation methods using a real data-driven simulation strategy as

we demonstrate here would provide useful insight as to whether imputation is a suitable

alternative to complete-case analysis.

As is often the case when constructing a complete-case dataset, several traits were

excluded from this study. These included many categorical traits that were invariant in the

complete-case dataset, such as those containing information about geography or habitat. In turn,

the range of phylogenetic signal for traits was also limited. It was therefore not feasible to truly

gauge the relationship between error ratio and phylogenetic signal strength in traits as they all

exhibited significant levels. The continued collection of high-quality trait data for both known

and novel species is necessary to further probe these types of relationships. For instance, in the

case of Squamata, snake species are disproportionally undersampled [9] and were thus not

included in the current study. An increase in data availability would also facilitate additional

research on the use of imputation methods in real datasets. Simulated trait data do not fully

capture the nuances of real datasets, and comparative evaluations using real data and different

taxonomic groups are needed to test whether imputing values is practical, particularly in cases of

biased missingness.

Missingness in datasets is a pervasive issue in the realm of biological research. It is

particularly problematic for those taxonomic groups threatened by extinction, or that are small or

reside in understudied areas of the globe. As trait data can take on many forms, methods that can

accurately predict missing values for diverse data types are instrumental for the study of these

obscure groups. Previous work has focused largely on numerical data, and consideration of

imputation performance for categorical traits is necessary to drive research in this field forward.

Every real trait dataset comprises a unique data structure shaped by both ancient and

contemporary processes, including the evolutionary history of the traits and existing sampling

biases. Consequently, some datasets may be more suitable for a particular imputation method

than others, especially so in the case of phylogenetic imputation. Researchers should therefore

take care to understand the properties of their dataset prior to and following imputation. Here, we

use a real data-driven selection strategy to select a best-suited imputation method for a given

target dataset. Simulating missingness using real data more accurately reflects the characteristics

and nature of the original data. Furthermore, our results suggest that, given a carefully selected

suitable imputation method, imputation can be used to bolster sample size while simultaneously

preserving the distributional properties of the original data. Derived inferences may then more

accurately represent the biological phenomena under investigation.

Materials and methods

Trait data

Traits are defined here as characteristics that are typical of a species. These may refer to

characteristics relating to the biology of a species or the environment in which it resides. Data for

squamates (lizards and amphisbaenians; order: Squamata) were selected for analysis as

complete-case observations were available for at least 100 species as well as both categorical and

numerical traits. In addition, these species had DNA sequence records publicly available for both

mitochondrial and nuclear markers. Squamata represent an incredibly diverse group of

vertebrates (~10,000 species) [54], inhabiting disparate environments and boasting a broad range

of morphological features. However, trait data for Squamata are undersampled relative to

mammal and bird groups, particularly in tropical regions that are home to diverse species at risk

[9]. As of 2022, 19.6% of squamate species are estimated to be under threat of extinction [55].

Imputation may offer additional avenues to identify those traits correlated with risk status in

squamates [56,57] and in doing so, contribute to biodiversity conservation efforts in vulnerable

areas. Trait data were obtained from a dataset published by Meiri [16,17] (other datasets were

also considered, see S1 File). This dataset contains information about the habitat, life history,

morphology, behaviour, and conservation threat level of 6,657 squamate species (lizards and

amphisbaenians, not including snakes) [16,17]. The raw trait data were downloaded into R v.

4.0.3 [58].

To assess which types of genetic information are useful for imputation, sequence data for

both mitochondrial and nuclear markers were used. The Barcode of Life Data System (BOLD)

[59] was used as the source for mitochondrial sequence data as it contains thousands of

published cytochrome *c* oxidase subunit I (COI) partial gene sequence records (16,676 sequences

for over 2000 Squamata species as of July 16th, 2021). COI barcode sequence data [60] for

Squamata were obtained from a published dataset [61], originally downloaded into R on March

12th, 2020. Data were filtered for records that have been identified to the species level, as this

information was necessary for trait matching purposes. Additional quality control checks on the

sequence data included trimming N and gap content from sequence ends and removing

sequences with greater than 1% of internal N and/or gap content across their entire sequence

length. Sequences between 650 and 1000 bp were retained to facilitate downstream multiple

sequence alignment. As multiple COI sequence records are available for many species, a

centroid sequence selection process was employed to find a typical representative sequence for

each species [62]. The centroid sequences used for multiple sequence alignment have been made

available at [63]. The *AlignTranslation* function from the R package "DECIPHER" v. 2.18.1

[64,65] was used to perform a multiple sequence alignment on the centroid sequences.

AlignTranslation was used as it performs a multiple sequence alignment guided by the translated

amino acid sequence, which is more reliable than an alignment based on nucleotide data alone

[64]. The translated final alignment was visualized using the *ggmsa* function from the R package

"ggmsa" v. 0.06 [66] to verify the nucleotides were in the correct reading frame and to check for

the presence of stop codons. The COI multiple sequence alignment and corresponding process

IDs are available on Dryad [67]. Nuclear sequence data were obtained from a multigene

alignment published in Pyron *et al.* [68,69]. This alignment is comprised of sequence data for 12

genes (seven nuclear, five mitochondrial) and 4161 species of Squamata [68]. The alignment was

partitioned into its constituent gene alignments using RAxML v. 8 [70].

To form a complete-case dataset for downstream missingness simulations, species names

from the COI alignment were first matched against the species names in the trait dataset. Those

species that had available data for at least five traits (both categorical and numerical) and a

corresponding COI sequence record were then matched against the species names in the nuclear

multigene alignment. The nuclear markers oocyte maturation factor (c-mos) and recombination

activating gene 1 (RAG1) had the largest number of available records for the species in the

complete-case dataset and were selected for analyses. Corresponding sequence identifiers of

those records selected (either BOLD process ID [59] or GenBank accession number [71])

available in S1 Table and on Dryad [67]. Final checks were performed on the trait data in the

complete-case subset. Categorical traits with severe class imbalances and very low variability

(e.g. more than 90% of observations in one of the categories and/or the remaining observations

sparsely dispersed across other categories), such as reproductive mode, geographic range, and

substrate, were excluded from the study. The distributions of numerical trait data were visualized

to check for the presence of severe outliers. For each numerical trait, an upper threshold was

calculated as follows: quartile $3 + (3 \times$ the interquartile range of the data). Severe outliers are

defined here as those values that exceed the upper threshold. If identified, these values were

verified in the primary literature to ensure they were real datapoints and not the result of data

entry error. The final dataset contains information for the seven most complete traits, including

the multi-categorical trait activity time, the binary trait insular endemic, the count traits: largest

clutch and smallest clutch, and the continuous traits female snout-vent length (SVL), maximum

snout-vent length (SVL), and latitude (geographic centroid for the species [72]). The final dataset

comprised 152 species, representing 25 Squamata families (S2 Table). To maintain a sufficient

sample size for missingness simulations, it was necessary to reduce the stringency for inclusion

of species in the complete-case dataset; otherwise, the sample size will drop to 121 if only

species without missing values in their traits are included. Consequently, we permitted some

missing values (no more than 10% for each trait) present in the so-called "near complete-case

dataset". To ensure enough data were available for each species for imputation, missing values

were only permitted in traits for species that had observed values for at least three of the other

traits in the dataset. For further details on these traits, see S3 Table.

Phylogenetic information
492

As high-quality sequence data may be unavailable for multiple markers for understudied

taxa, one of our objectives was to demonstrate the utility of single gene trees for imputation. The

alignments for the COI, c-mos, and RAG1 sequences were used to build maximum likelihood

gene trees in RAxML v. 8 [70]. For comparison purposes, a composite gene tree was also built

using a combined multigene alignment of the COI, c-mos, and RAG1 sequences (referred to as

"MG" for multigene). The model GTRGAMMAI was specified (option -m), and the alignments

were partitioned based on codon position (option -q). The trees were then read into R and made

ultrametric using the *chronos* function in the R package "ape" v. 5.4.1 [73]. Phylogenetic

eigenvectors were extracted from each tree and for each trait using the "MPSEM" package v.

0.3.6 in R [74]. The number of eigenvectors that explained greater than or equal to 65% of the

phylogenetic structure variance was used (see S1 File for further details on this process).

Following the method of Penone *et al.* [11], the phylogenetic eigenvectors were appended to the

near complete-case dataset and treated as predictors in the model to impute the missing value of

a given trait. All trees used in the phylogenetic imputation process have been made available on

Dryad [67].

Previous studies have suggested that phylogenetic signal strength in simulated trait data is

positively correlated with imputation accuracy [32,75]. To assess this association using real data,

we measured phylogenetic signal for each trait using Pagel's λ [42] for numerical traits and the *D*

metric [41] for categorical traits. Pagel's λ is estimated using maximum likelihood and represents

the value that optimally transforms a phylogenetic variance-covariance matrix to fit the observed

trait data structure. A λ value of 0 indicates no phylogenetic signal (star-shaped phylogeny),

whereas a λ value of 1 suggests that the trait data adhere to a Brownian motion (BM) model of

evolution [42]. The *D* metric represents whether the number of transitions of a binary trait varies

from the expected number under a BM model [41]. A *D* value of 0 indicates that the trait data

adhere to a BM model, and a *D* value of 1 indicates that there is no phylogenetic signal in the

trait data. A *D* value greater than 1 signifies phylogenetic overdispersion. Alternatively, a *D*

value less than 0 suggests the trait is phylogenetically conserved [41]. These metrics were

calculated separately for each trait using each gene tree (S1 File). The *phylosig* function in the R

package "phytools" v. 0.7.70 [76] and *phylo.d* function in the R package "caper" v. 1.0.1 [77]

were used to measure λ and D, respectively.

Candidate imputation methods

The performances of four candidate imputation methods were considered: mean/mode

imputation, *k*-nearest neighbour (*KNN*) ("VIM" package v. 6.1.0) [24], random forests (*RF*)

("missForest" package v. 1.4) [40,78], and multivariate imputations by chained equations

(*MICE*) ("mice" package v. 3.13.0) [27]. Mean (for numerical traits) / mode (for categorical

traits) imputation, the simplest method, was used as a baseline for comparison. The remaining

methods were chosen due to their popularity in trait-based studies [35,79] and capacity to impute

both numerical and categorical traits. These methods have also been evaluated in previous

studies of trait data imputation [11,34,44]. *KNN* and *RF* are single imputation methods as they

provide a single estimation of the missing value. *MICE* is a multiple imputation method that

performs imputation *m* times on the dataset with missing values, resulting in *m* imputed datasets.

The *MICE* algorithm utilizes chained equations to estimate missing values and offers several

different models for imputing data. In this study, the predictive mean matching model was used

to estimate missing continuous data. Predictive mean matching is the default model for

continuous data in *MICE* and performed well in previous evaluations using trait data [44,80].

Predictive mean matching fills the missing observation with a random value selected from a

"donor" pool for the missing observations. This pool is created by fitting a regression model on

the observed data and selecting *k* fitted values that are closest to the predicted value for the

missing observation [45,46]. Logistic regression is a common approach for predicting missing

binary data and is the default method for data of this type in *MICE*. Logistic regression and

polytomous logistic regression models were thus used to impute values for the binary trait insular

endemic and the nominal multi-categorical trait activity time, respectively. To obtain a final

imputed value for *MICE*, the mean and mode values were taken across the *m* datasets for

numerical traits and categorical traits, respectively. See S1 File for further details on imputation

algorithms.

Missing values of each trait ("target trait") are imputed using values of the other traits

("auxiliary traits"); however, not all of the auxiliary traits are useful for imputation. Association

tests between each pair of traits were used to filter out irrelevant auxiliary traits and build a more

parsimonious imputation model for the target trait. Regression models were used in the

association tests in which the target trait was specified as the response variable and each one of

the auxiliary traits was specified as the covariate. Linear regression, Poisson regression, logistic

regression, and polytomous logistic regression models were used for continuous, count, binary,

and multi-categorical target traits, respectively. Only auxiliary traits with a coefficient not

significantly equal to zero were retained in the imputation model for a particular target trait.

Finally, as methods such as *KNN* are sensitive to the range of the data, numerical traits were

natural log-transformed prior to imputation.

Real data-driven simulation strategy

Missingness simulations

The first step in the real data-driven simulation strategy was to simulate missing values in

the near complete-case dataset (Fig 7). Three different missingness mechanisms were

considered: 1) missing completely at random ("MCAR"); 2) missing at random ("MAR"); and 3)

missing not at random ("MNAR"). Under the MCAR mechanism, missing values were randomly

introduced into the near complete-case dataset at different proportions (0.10, 0.20, 0.30, and

0.40). In cases where traits had values that were already missing (up to 10%), missing values

were introduced on top of these (i.e. up to 50% missingness). To reduce stochasticity and

maintain a fair comparison of imputation performance across different missing proportions, and

not introducing variability relating to species identity, missing data for each increase in

proportion (e.g. from 0.10 to 0.20 missingness) were added upon the missing values of the

previous proportion. To simulate MAR using real data, logistic regression models were fitted to

the original Meiri [16] dataset ($n = 6657$) to identify which auxiliary traits were significantly

associated with the missingness for each target trait. In the fitted model, the indicator of whether

an observation is missing or not was treated as the response variable and auxiliary traits specified

as predictors. The fitted models were then used to introduce missing values into the near

complete-case dataset (for further details see S1 File). To test how the imputation methods

perform in cases of extreme bias, MNAR scenarios were simulated for each trait. Values were

removed from the 10th percentile of the tail of data distribution for numerical biological traits,

e.g., the 10th percentile of the lower latitudes (between 10° and -10°); and from a single category

for categorical traits, e.g., "nocturnal" category for activity time and "yes" category for insular

endemic. These values were removed to emulate realistic MNAR scenarios for Squamata (see S1

File for further information).

Fig 7. Workflow of the real data-driven simulation strategy. 1) Missing values are simulated

in the near complete-case dataset under MCAR, MAR, and MNAR mechanisms. Missing dataset

refers to the near complete-case dataset with simulated missing values; 2) imputation of

simulated missing values using different candidate methods (with or without phylogeny); 3)

identification of the best-suited method based on the performances (MSE and PFC) of the

candidate methods. The imputed values from 2) are compared to the true observed values in the

near complete-case dataset to derive MSE and PFC for numerical and categorical traits,

respectively. Note that the "true observed values" refer to the trait values reported in the source

Meiri (2018) dataset [16,17]. These values are assumed to contain a negligible amount of

measurement error in this context and provide a close representation of the true biological values

for squamates. MSE and PFC are averaged across 100 replicates; and 4) application of the best-

suited imputation method to the original target dataset with missing values. Original data,

original data with imputed values, and complete-case data characteristics and distributions are

compared to assess how imputation impacts dataset properties.

Imputation using candidate methods
601

The second main step in the strategy was to apply each candidate method to impute the

simulated missing values under the different simulation settings. A range of parameters and their

values were considered for the different candidate imputation methods (see S1 File for details on

this process). The parameters that resulted in the lowest error rate were used in the imputation

model. Imputations using only trait data were first performed on the simulated missing dataset.

Imputations were again performed using trait data and phylogenetic eigenvectors derived from

either COI, RAG1, c-mos, or MG trees. This amounted to 96 different combination settings with

respect to method and missingness mechanism. The entire process was repeated 100 times for

each combination of settings, resulting in 9,600 runs of the simulation and imputation pipeline

strategy.

Performance evaluations
613

The third main step in the strategy was to evaluate the performances of each candidate

method under the different simulation settings. Imputation performance is defined in this context

as how well (i.e. accurately) the candidate method predicts the true observed values. The "true

observed values" refer to the values as reported in the Meiri (2018) dataset [16,17]. It is assumed

that these values are close to the true biological values for squamates and that the error in the

reported values is generally small in comparison with the total variability in each trait across all

species in the dataset. To assess performance, mean squared error (MSE) rates and proportion

falsely classified (PFC) rates were computed for numerical and categorical traits, respectively.

To compute MSE and PFC, only those imputed values that were simulated as missing in the near

complete-case dataset and that have true observed values were used. For both metrics, values

closer to 0 are indicative of better performance. To illustrate the impact of missingness

percentage on imputation accuracy, we generated an equal number of missing data replicates for

each missingness percentage (10%, 20%, 30%, 40%). As other simulation settings (missingness

mechanism, imputation method with or without phylogenetic information) are preserved for each

missingness percentage, this enables a fair comparison of imputation accuracy impacted by

missingness percentage. Vice versa, the performance of imputation using different methods can

be compared fairly using replications of the same missingness percentage. The performance

metrics (MSE and PFC) were averaged across the 100 replicates for each combination of

methods for each trait. The packages "ggplot2" v. 3.3.5 [43] and "plotly" v. 4.10.0 [81] were

used to visualize results in R. The best-suited method is defined here as the imputation method,

either with or without phylogeny, that resulted in the lowest average error rates (either MSE for

numerical traits or PFC for categorical traits) for the most traits (i.e. "a majority vote"). To select

the best-suited method, the results of the MAR simulations were first considered as these mimic

realistic biological scenarios. In case of more than one method performing similarly under MAR

(e.g. a tied vote), the results of MNAR and MCAR were also considered.

Application of best-suited imputation method to original dataset
640

The fourth main step in the strategy was to apply the best-suited imputation method to the

original target dataset and compare the characteristics between the original data, original data

with imputed values, and complete-case data for each trait. To assess the influence of imputed

values on data characteristics, summary statistics for each trait were calculated using the original

dataset with imputed values and qualitatively compared with the summary statistics of both the

original and complete-case data. To investigate whether the phylogenetic information improves

the imputation accuracy for a given trait and imputation method, the following error ratio was

calculated for each trait and each method:

$$Error\ ratio = \frac{Error\ rate\ (MSE\ or\ PFC)\ without\ phylogeny}{Error\ rate\ (MSE\ or\ PFC)\ with\ phylogeny}$$

An error ratio value greater than 1 indicates an improvement in imputation performance resulting

from the addition of phylogenetic information. To observe the trend of the effect of phylogenetic

signal strength on the imputation of different traits, the error ratio values were plotted against the

λ and D metrics for numerical and categorical traits, respectively.

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Supporting information

S1 File. Supplementary Information.

S1 Fig. Phylogenetic signal measurements. Measures of phylogenetic signal for a) categorical

and b) numerical traits in gene trees constructed for mitochondrial COI and nuclear c-mos and

RAG1. Asterisks indicate significance at the 0.05 level, according to results from hypothesis

tests comparing the results to a null model (no phylogenetic signal). Fritz and Purvis' *D* metric

[41] and Pagel's λ [42] were used to measure phylogenetic signal for categorical and numerical

traits, respectively. In the case of *D,* lower values are indicative of higher levels of phylogenetic

conservation for the trait; conversely, higher values of λ suggest stronger phylogenetic signal. As

the *D* metric only measures the phylogenetic signal of binary traits, the three-level categorical

trait AT was broken down into the binary traits "AT: Diurnal" and "AT: Nocturnal".

S1 Table. Sequence identifiers.

S2 Table. Taxonomic composition of near complete-case trait dataset (n = 152).

S3 Table. Descriptions and additional details for traits in the near complete-case dataset.

Proportion of present data (n = 8219)