

No news from old drawings? Stomach anatomy in muroid rodents in relation to body size and ecology

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Abstract

Muroid rodents mostly have a complex stomach: one part is lined with a cornified (nonglandular) epithelium, referred to as a “forestomach”, whereas the rest is lined with glandular epithelium. Numerous functions for the forestomach have been proposed. We collated a catalog of anatomical depictions of the stomach of 174 muroid species from which the respective nonglandular and glandular areas could be digitally measured, yielding a “stomach ratio” (nonglandular:glandular area) as a scale-independent variable. Stomach ratios ranged from 0.13 to 20.15, and the coefficient of intraspecific variation if more than one picture was available for a species averaged at 29.7% (± 21.5). We tested relationships of the ratio with body mass and various anatomical and ecological variables, including diet. There was a consistent phylogenetic signal, suggesting that closely related species share a similar anatomy. Apart from classifying stomachs into hemiglandular and discoglandular, no anatomical or ecological measure showed a consistent relationship to the stomach ratio. In particular, irrespective of statistical method or the source of dietary information, dietary proxies did not significantly correlate with the stomach ratio, except for a trend towards significance for invertivory (insectivory). Yet, even this relationship was not convincing: whereas highly insectivorous species had high but no low stomach ratios, herbivorous species had both low and high stomach ratios. Thus, the statistical effect is not due to a systematic increase in the relative forestomach size with invertivory. The most plausible hypotheses so far associate the muroid forestomach and its microbiome with a generic protective role against microbial or fungal toxins and diseases, without evident correlates of a peculiar need for this function under specific ecological conditions. Yet, this function remains to be confirmed. While providing a catalog of published depictions and hypotheses, this study highlights that the function of the muroid rodent forestomach remains enigmatic to date.

KEYWORDS

forestomach, gastrointestinal tract, Myomorpha, Rodentia

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1 | INTRODUCTION

The Rodentia is a highly diverse order of mammals present almost everywhere on earth (D'Elía et al., 2019). Most likely, their ancestral trophic level was that of omnivores or insectivores (Breed & Ford, 2007; Landry, 1970), although today, rodents exhibit a broad range of diets from herbivores to faunivores. This variety is also present within the most speciose rodent superfamily, the Muroidea (Gainsbury et al., 2018; Wilman et al., 2014).

Among other factors, the Muroidea differ from other rodents in their stomach anatomy (Langer, 2017). Muroid stomachs are generally composed of a section with cornified (nonglandular) squamous epithelium, and a section of glandular epithelium. The nonglandular part is close to the cardia and the glandular part close to the pylorus, suggesting a sequence of ingesta first entering the nonglandular part before being exposed to the gastric secretions. The transition between the two regions is typically abrupt, represented by a bordering fold (or limiting ridge) lined with cornified epithelium (Figure 1). The glandular region consists of varying proportions of cardiac, fundic, and pyloric glands. For a detailed compilation of histological descriptions of the muroid stomach, see Supporting Information: Table S1. In some species, the nonglandular epithelium is papillated (Langer, 2017).

The macroscopic conformation of the stomach lends itself to different classifications (Carleton, 1973; Langer, 2017). In some species, the stomach appears as one bag-like compartment that is only separated into the two regions internally, suggesting the label of a “unilocular” stomach (Figure 1a,b). Alternatively, the stomach may be sequestered into two or (rarely) more compartments by strictures

or incisurae (in particular, the *Incisura angularis*), suggesting the label of a “bilocular,” “multilocular,” or (multi)chambered stomach (Figure 1c,d). The stricture need not necessarily conform to the border of the two epithelial regions (Figure 1d), and it can be more or less distinct, making the classification between unilocular and bilocular stomachs somewhat arbitrary. Possibly one of the most complex muroid stomachs is that of the African maned or crested rat *Lophiomyys imhausi* with four different chambers (Naumova & Zharova, 2003). Additionally, the glandular region of the stomach may represent a large part of the distal stomach, which is then described as “hemiglandular” (Figure 1a,c). Alternatively, the glandular region may be constrained to a disc-shaped, smaller section of the distal stomach that need not be in close vicinity of the pylorus. These stomach types have been named “discoglandular” (Figure 1b,d). Different combinations of these stomach types may occur, giving rise to a large anatomical variety that has been documented for a long time (Langer, 2017). The “hemiglandular” stomach description has been expanded to comprise a “sub-hemiglandular” label for stomachs in which the glandular part is smaller, yet does not represent the “discoglandular” conformation (Langer, 2017). If the opening of the “discoglandular” part into the nonglandular stomach is narrow, sequestering the glandular part, then the label “diverticular” can also be applied (Langer, 2017).

Especially in the case of a distinct stricture with a clearly separated nonglandular region, but also in the case of an expansive nonglandular region in a unilocular stomach type, this compartment is conveniently interpreted as a “forestomach.” Similar to the forestomach of larger herbivores, a gastric “groove” has been described in some muroid forestomachs that is interpreted to aid in channeling

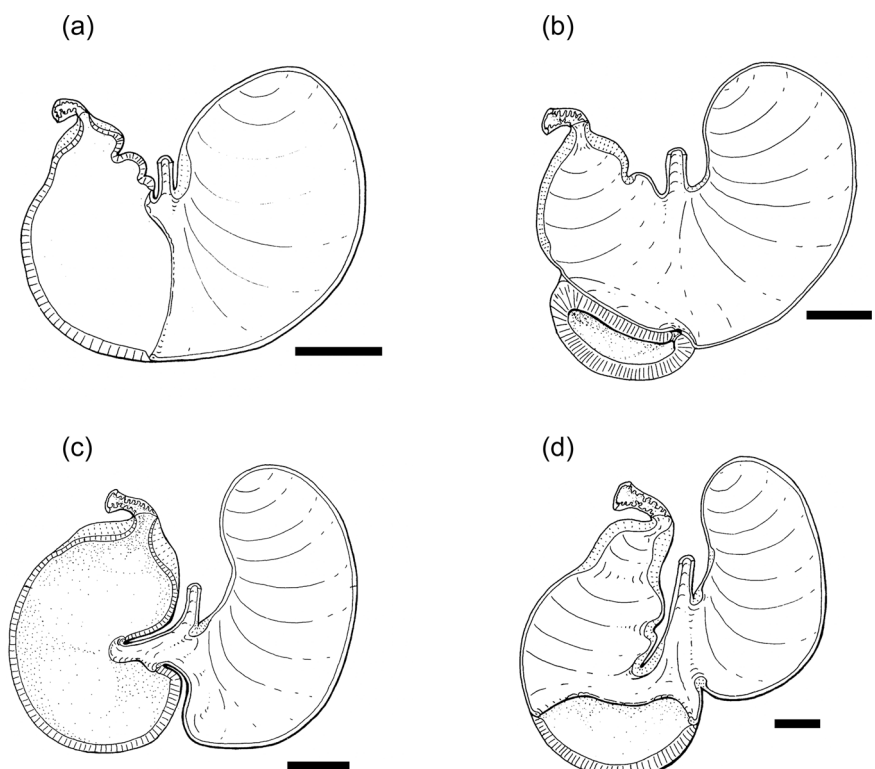


FIGURE 1 Illustration of different muroid stomach types from Carleton (1973), with (a) a unilocular/hemiglandular type (*Oxymycterus rutilans*), (b) a unilocular/discoglandular type (*Reithrodontomys mexicanus*), (c) a bilocular/hemiglandular type (*Cricetus cricetus*), and (d) a bilocular/discoglandular type (*Neotoma phenax*). Nonglandular epithelium indicated by broadly spaced dashes. The scale bar in each graph represents 5 mm.

milk in suckling juveniles past the forestomach section, including species with a discoglandular stomach (Langer, 1993). This seems to contrast with the hypothesis of Genest-Villard (1968) that in some species with a discoglandular stomach, the nonglandular compartment may not serve any purpose in adult individuals but as a receptacle for large amounts of milk, supporting particularly high juvenile growth rates.

The function of a forestomach is considered well-understood in the case of large herbivores from a variety of mammalian groups, such as kangaroos, sloths, colobine monkeys, peccaries, hippopotami, camelids, and ruminants (Stevens & Hume, 1998); it is generally seen in hosting a microbiome that aids in fermentative plant cell wall digestion (and at the same time represents a source of protein for the host once the microbes are flushed out of the forestomach into the lower digestive tract).

By contrast, the function of the muroid forestomach is not well understood. Supporting Information: Table S2 gives a detailed bibliography on speculations and investigations on the function of the muroid forestomach. In terms of mechanical functions, the forestomach has been suggested to represent a storage organ for food or water, a compensation for a lack of cheek pouches, a site where potentially abrasive, sharp, or—in the case of insects—putatively still biting food is stored before digestion, or a gizzard-like structure that contributes to particle size reduction of the ingesta. Whereas the generic function of ingesta storage cannot be refuted (yet does not require a peculiar anatomical structure, as most simple stomachs can fulfill the same function), the other suggestions appear unlikely (Supporting Information: Table S2). The forestomach has also been suggested to represent a location still devoid of gastric secretions, which would allow a more intensive pre-gastric digestion of the ingesta by salivary enzymes; while this theory focuses mostly on salivary amylase, hypothetical action of salivary lipase and chitinase action have similarly been suggested (Supporting Information: Table S2). The analytical difficulty in testing this assumption is the differentiation between salivary and microbial enzyme action.

This is because the forestomach harbors a microbiome; whenever this has been investigated, the presence of microbes has been confirmed (Supporting Information: Table S2). Thus, most hypotheses on the adaptive value of the muroid forestomach revolve around the microbiome's function. The original assumption of plant cell wall fermentation, as in other herbivorous foregut fermenters, has been refuted by more detailed analyses of the microbiome, which is mostly comprised of lactic acid-producing bacteria and not of microbes capable of fiber fermentation (Supporting Information: Table S2). The value of the microbiome might lie in the detoxification of specific secondary plant compounds (Kohl et al., 2014). Lactic acid-producing bacteria, including lactobacilli, protect against fungi and fungal mold (Oliveira et al., 2014), and also suppress the growth of enteropathogenic microbes (Jena et al., 2013). This would make the muroid rodent forestomach a generally protective adaptation, and as an explanation of its morphological

variety, specific requirements of such protection in specific ecological situations would have to be hypothesized.

In this study, we aim to use the published drawings on muroid rodent stomachs to derive a quantitative measure of forestomach size, by calculating the area of nonglandular to glandular epithelium following Perrin and Curtis (1980) and linking the resulting ratio to a variety of biological and ecological characteristics of the respective species in an explorative approach. In particular, we followed the hypothesis laid out in Shinohara et al. (2016) and Langer and Clauss (2018) that species with a higher proportion of seeds in their natural diet, and species that use food hoarding, should particularly benefit from the anti-fungal properties of a lactic acid-producing forestomach microbiome and should therefore have particularly well-developed nonglandular stomach compartments.

2 | MATERIAL AND METHODS

2.1 | Literature illustration collection

The data set (Supporting Information) used for this study includes 174 species of muroid rodents. The species were selected opportunistically based on the availability of a published illustration of the stomach from which the area of the nonglandular and glandular epithelium could be assessed in sagittal view (corresponding to Figure 1). The search for literature sources was primarily guided by major works on rodent or mammal gastrointestinal anatomy (Carleton, 1973; Langer, 2017; Vorontsov, 1967) that provided both original data or indicated original sources. Additionally, a web-based literature research was performed using Google Scholar and combinations of “rodent,” “Rodentia,” “stomach,” “forestomach,” “anatomy,” and specific genus names as search words, and again literature cited in, or citing, publications thus collated. Illustrations were obtained from a total of 70 sources and are shown in the Supplementary online material. Illustrations were not used for measurements if no clear discrimination between the different epithelia was possible but were nevertheless included in the catalog. While we are unaware of additional literature, we do not claim that our species catalog is exhaustive. In cases where more than one depiction was given in the same source, both images were used, and differences were hence implied to represent “natural variation” or “methodological variation.” In cases where it appeared evident that a graph represented the copy of a graph of a previous publication, regardless of whether it was cited or only looked identical, the second image was not used (i.e., no replication of measurements for identical images). However, this approach bears some subjectivity, as we could not rule out that slightly varied representations (i.e., freely re-drawn without referencing) were included in the data set. For a single species (*Hydromys chrysogaster*), two independent sources showed a similar anatomy by drawing and photograph, whereas a third, much older source depicted a completely different anatomy by schematic drawing. In this case, the deviating source was excluded

from the measurements. All decisions are indicated in the image catalog.

Using the illustrations collected from the literature, the areas represented by the nonglandular and the glandular epithelium in a sagittal view were measured using Adobe Photoshop 2020 (see Supporting Information for details), following the respective contours on the illustrations. Measurements were made with the default setting using pixel as a scale (scale: 1 pixel(s) = 1.0000 pixels, scale units: pixels, scales factor: 1.000000). Structures such as the esophagus, duodenum, and borders of the illustration such as thick epithelial layers or muscle layers were left out from the measurements. The ratio of nonglandular: glandular epithelium area was calculated for every illustration, making this ratio a body-size independent measure. For each species, the mean ratio of the individual ratios of accepted illustrations was calculated, as well as the standard deviation and the coefficient of variation if more than one illustration for the species was measured. Based on the illustrations and descriptions, the stomachs were classified as having a papillated or nonpapillated nonglandular region, of having a deep *I. angularis* or not, and of being of the hemiglandular or the discoglandular type (Figure 1). For some of the graphical illustrations of the data set, stomachs were categorized into five groups (Group 1 including ratios from 0 to 0.99, Group 2 from 1 to 1.99, Group 3 from 2 to 5.99, Group 4 from 6 to 9.99, and Group 5 from 10 to 25); for the corresponding statistics, however, the stomach ratio was used as a continuous variable.

2.2 | Biological and ecological species traits and phylogenetic information

Several biological and ecological characteristics were added to the species list from various sources. These included body mass (g), mean latitude, actual evapotranspiration rate (AET) (mm), precipitation (mm) (Jones et al., 2009), natural diet composition as the percentage of faunivory, invertivory, herbivory, granivory, frugivory, and folivory in natural diet (Wilman et al., 2014), food hoarding behavior (no/yes) (McCarty & Southwick, 1975; Miljutin, 2011) and the occurrence of cheek pouches (no/yes) (Miljutin, 2011; Ryan, 1989; Vander Wall & Dittel, 2021) or birth seasonality (Heldstab, 2021). Additionally, data on body mass and intestine lengths (small intestine, large intestine, cecum, total GIT length; Duque-Correa et al., 2021) and body mass and basal metabolic rate (BMR; Genoud et al., 2018) were used for models that used log-transformed intestine lengths or BMR as the dependent variable and log-transformed body mass as the independent variable, to test whether the addition of the stomach ratio as a covariable increased the data fit of these models. Note that not all information was available for all species.

Because the analyses using the diet data from Wilman et al. (2014) did not yield the expected results, we performed a literature search for original quantitative diet information for the species represented in our data. In this way, original data for 67 species were gained from the literature, using the same hierarchical classifications

as for the Wilman et al. (2014) data set, where invertivory is included in faunivory, and herbivory comprises the categories of granivory, frugivory, and folivory. In cases where multiple sources were available for a species, we calculated both, the mean and the maximum value for each diet category. To test the agreement between both dietary proxies, we used Spearman's correlation.

The phylogenetic tree was built following Upham et al. (2019). A consensus supertree inclusive of 5911 mammalian species with time calibration was downloaded from (<http://vertlife.org/phylosubsets/>). The supertree was pruned in R using scripts from the library "ape" (Paradis et al., 2004) and "tidyverse" (Wickham et al., 2019) to obtain a final tree inclusive only of the 174 species of the present study. This tree is also given in the Supporting Information. Using the R package *ggtree* version 3.2.1. (Yu et al., 2017), the biological and morphological characteristics were visualized by mapping them onto a phylogenetic tree including all species for which a stomach ratio was available.

2.3 | Statistical analyses

Analyses were performed using generalized least squares (GLS) and phylogenetic generalized least squares (PGLS), recording the 95% confidence interval for parameter estimates, using the R packages "caper" (Orme et al., 2013) and "nlme" (Pinheiro et al., 2016). In all PGLS models, phylogenetic signal, lambda (λ) was estimated by maximum likelihood. One set of models used the stomach ratio as the dependent and either body mass (alone or with an additional dichotomic or diet factor) or another continuous variable as the independent variable. For intestine lengths and BMR, length or BMR was the dependent variable, the corresponding body mass the independent variable, and the stomach ratio the co-variable. In all models, stomach ratio, body mass, intestinal lengths, and BMR were used log-transformed. The significance level was set to 0.05. To compare model fit irrespective of the statistical significance of individual variables, we additionally used the small sample corrected Akaike's information criterion ($\Delta AIC_c > 2$) as providing a different fit to the data (Burnham & Anderson, 2002).

2.4 | Additional dissections

To explore the magnitude of what might be considered an acceptable coefficient of variation for measurements within a species, the stomachs of 10 laboratory rats (Wistar, 12-week-old, male) and 10 laboratory mice (CD1, female 12-week-old) were dissected. The animals were euthanized at the end of another experiment (experimental animal license ZH243/2019) without any impact on the gastrointestinal tract and were previously maintained on a commercial laboratory animal feed. An incision was made along the greater curvature, the stomach was opened, the contents removed, and one side of the stomach was laid out flatly and photographed (Supporting

Information: Figure 1). The processing of the photographs was identical to the anatomical drawings. For each species, the coefficient of variation of the stomach ratio was calculated.

3 | RESULTS

3.1 | Stomach ratio—descriptives

Our own measurements on 10 laboratory rats and mice each yielded a mean stomach ratio of 0.79 (± 0.27) and 0.61 (± 0.22), respectively, with a coefficient of variation of 34% for rats and 37% for mice (Supporting Information: Table S3). For rats, our own measurements resembled the ratio of 0.75 from the drawing in Hebel (1969), and our coefficient of variation was similar to that across the measurements of all published rat drawings at 36%, but the average stomach ratio gleaned from published drawings for rats was, at 1.24 (± 0.45), distinctively higher than our own value. For mice, the situation was similar; our own measurements resembled the ratio of 0.77 from the drawing in Nachtigal (1987), and our coefficient of variation was similar to that across the measurements of all published drawings at 38%, but the average stomach ratio gleaned from published drawings for mice was, at 1.28 (± 0.49), distinctively higher than our own value.

Illustrations that could be used for measuring the stomach ratio were available for 174 muroid species (Supporting Information). Of a total of 312 illustrations that could be used, 29 most likely represented (modified) copies from an older illustration, resulting in a total of 283 illustrations used.

The species-specific average stomach ratio ranged from 0.13 (*Pseudomys gracilicaudatus*) to 20.15 (*Peromyscus mexicanus*). For species with more than one illustration, the coefficient of variation averaged 29.7% (± 21.5).

3.2 | Comparative analyses

3.2.1 | Phylogenetic signal

The results of PGLS analyses indicated a certain degree of phylogenetic signal in most analyses, with λ generally at about 0.6–0.7 (Supporting Information: Tables S4–10), suggesting that closely related species share a similar stomach anatomy. This was also evident when plotting the stomach ratio onto the phylogeny (Supporting Information: Figure S2).

3.2.2 | Body mass and anatomy

In GLS, there was a negative relationship between the stomach ratio and body mass, suggesting relatively larger forestomachs at smaller body sizes (Figure 2; Supporting Information: Table S4). However, this relationship was not significant at PGLS (Supporting Information:

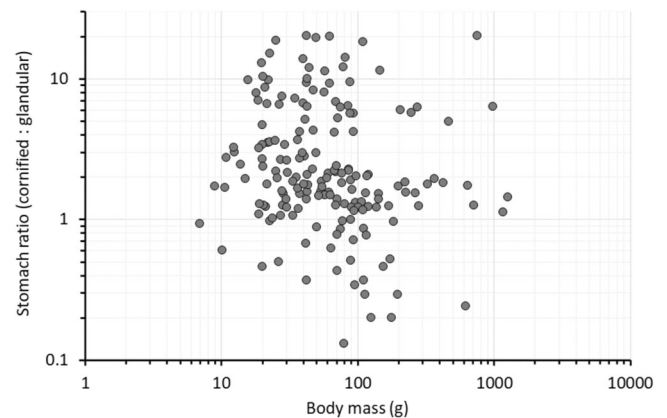


FIGURE 2 Relationship of the stomach ratio (area of cornified: glandular epithelium) with body mass in 174 muroid rodent species. For statistics, see Supporting Information: Table S4.

Table S4), indicating that forestomach size did not decrease with body size within lineages, but that larger lineages generally had smaller forestomachs.

For four species, reports and illustrations indicated a forestomach lined with papillae (*Cricetomys gambianus*, *Myospalax myospalax*, *Mystromys albicaudatus*, and *Tachyoryctes splendens*, for further details, see Supporting Information). These species did not stand out in terms of their stomach ratio (Supporting Information: Table S4, Supporting Information: Figure S3A). Stomachs with a deep angular incision had a higher stomach ratio in GLS but not in PGLS, again indicating that both characteristics were unevenly distributed across lineages and did not change systematically within lineages (Supporting Information: Table S4, Supporting Information: Figure S3B). Stomachs considered to have a disc-shaped glandular area had a distinctively higher stomach ratio, regardless of whether this was assessed in GLS or PGLS (Supporting Information: Table S4, Supporting Information: Figure S3C), suggesting that this stomach type evolved repeatedly in different muroid lineages and is consistently associated with a reduced glandular region. At 0.2, λ was low in models including this anatomical feature, suggesting it is not particularly constrained to a phylogenetic lineage but evolved multiple times in different lineages (Supporting Information: Figure S4). The model including the presence of a disc-shaped glandular area had the best support, also outranking the one with body mass only (Supporting Information: Table S4). When both the presence of an angular incision and a disc-shaped glandular area were included in the model, the incision was not significant (Supporting Information: Table S4).

The stomach ratio did not have a significant effect on the body mass–intestine length relationships for any intestinal section, also not when accounting for diet (Supporting Information: Table S5, Supporting Information: Figure S5). There was no relationship with the presence of cheek pouches, even though the model including it did not have less support than the model using only body mass (Supporting Information: Table S6, Supporting Information: Figure S6).

3.2.3 | Natural diet

Using the natural diet indicated by Wilman et al. (2014), no diet category was significantly related to the stomach ratio, and the body mass-only model had the best support in GLS (Supporting Information: Table S7, Supporting Information: Figure S7). In PGLS, the models using invertivory (with a positive yet not significant effect), folivory (with a positive yet not significant effect), and body mass only were equally supported (Supporting Information: Table S7), with invertivory tending towards significance in PGLS ($p = .069$); neither graph indicates a relevant pattern (Figure 3a,b). For invertivory, the graph shows that species that do not eat invertebrates can have both, low and very high stomach ratios. Thus, the graph suggests that the statistical effect is rather due to an absence of species in which the forestomach is smaller than the glandular stomach (a ratio <1) among the insectivores rather than a systematic increase in the relative forestomach size with invertivory (Figure 3b).

For 67 species, original quantitative data on the natural diet were available from the literature (Supporting Information). Compared to the data from Wilman et al. (2014), these data were largely in discord, with the highest Spearman's correlation coefficient not surpassing 0.56 (Supporting Information: Table S8). Using these data, the single best-supported model in GLS was the body mass-only model, even though invertivory was significantly related to the stomach ratio (Supporting Information: Table S9). In PGLS, the body mass-only model was among the best supported, and some of the diet categories were also included in models of similar support, yet none of the diet categories were significant. However, at a p of 0.054, invertivory again tended towards significance and was included in the model of the lowest AIC value (Supporting Information: Table S9). Again, the graph shows that species that do not eat invertebrates can have both, very low and very high stomach ratios, and thus suggests that the statistical effect is due to an absence of species in which the forestomach is smaller than the glandular stomach (a ratio <1) among the invertivores rather than a systematic increase in the relative forestomach size with invertivory (Supporting Information: Figure S8).

3.2.4 | Habitat and aspects of seasonality

Measures of habitat aridity (AET, precipitation) were not significantly correlated to the stomach ratio (Supporting Information: Table S10, Supporting Information: Figure S9A,B). There was no relationship between the stomach ratio and the mean latitude of origin (Supporting Information: Table S10, Supporting Information: Figure S9C). There was no relationship between reproductive seasonality and the stomach ratio (Supporting Information: Table S10, Supporting Information: Figure S9D) or whether a species was considered to practice food hoarding or not and the stomach ratio (Supporting Information: Table S10, Supporting Information: Figure S9E).

3.2.5 | BMR

In the body mass–BMR relationship, the stomach ratio had a significant additional effect in GLS but not in PGLS (Supporting Information: Table S11). In both analyses, the stomach ratio did not lead to notably better-supported models. An inspection of the respective graph does not indicate a clear pattern (Supporting Information: Figure S10).

4 | DISCUSSION

The main conclusion of the present study is that a set of comparative analyses did not yield a result that could shed light on the function of the muroid forestomach. Assuming that the data used in the present study reflect real anatomical proportions, it seems that there is little evidence for convergence to point towards an adaptive function of this organ. Even the one finding that had some statistical support, irrespective of which of the two different diet datasets used—a link with invertivory—does not yield a compelling visual pattern, and relatively large forestomachs occur in both invertivorous and noninvertivorous animals.

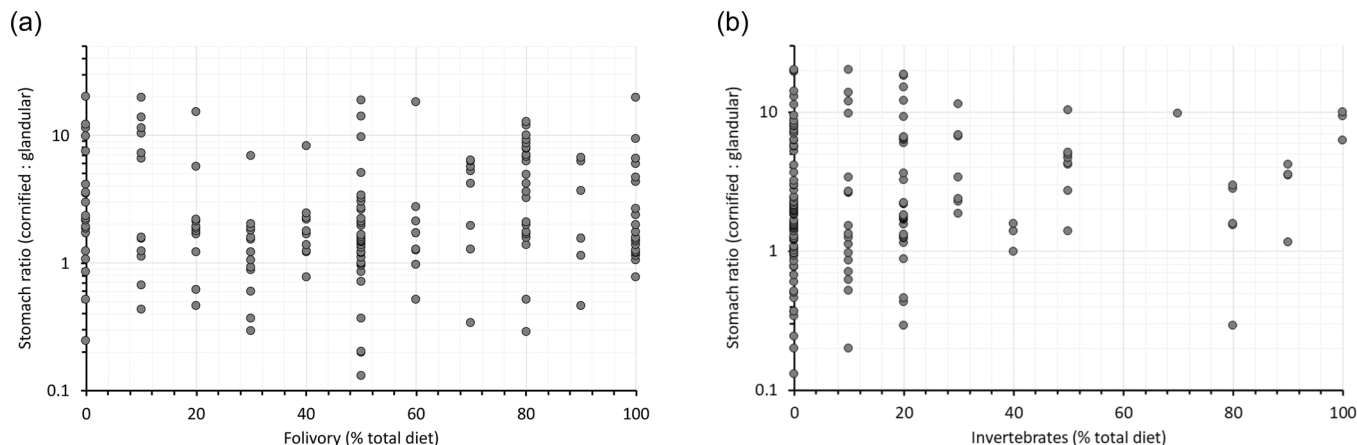


FIGURE 3 Relationship of the stomach ratio of 174 muroid rodents with either (a) the percentage of folivory or (b) the percentage of invertivory according to Wilman et al. (2014). For statistics, see Supporting Information: Table S7.

Therefore, it may be particularly interesting to consider several constraints of the present study. We did not use images that appeared—admittedly, mostly due to our subjective judgment and not due to a citation—to be copies of previously published graphs. Thus, we ensured that the coefficient of variation was not unduly reduced by repeated inclusion of the same depiction. We used anatomical depictions from various sources, none of which reported a detailed dissection method that would facilitate an exact replication of the approach. In theory, this must be prone to inherent methodological variation. For example, different states of the investigated material (fresh vs. stored in formalin or alcohol) might yield different results—a question we failed to address in our own dissections. But one could also argue that the way of depiction—of a stomach laid on its side, with the top half of its wall taken off, is in itself comparatively standardized even without textual descriptions. Another source of variance will be naturally occurring variation in forestomach and glandular stomach size within a species, possibly also due to differences in the filling state of the stomach at the point of death of the specimens. The fact that the coefficient of variation in the measurements we performed ourselves during a single dissection session in two species resembles that of the different species-specific measurements taken from the literature is somewhat reassuring and lends support to the assumption that the variation in the literature graphs resembles a naturally occurring one and need not be due to differences in methods. However, the difference in our own rat and mice measurements to the averages we gleaned from the literature for these species either suggests that natural variation within a species can be substantial, or that the reliability of published drawings, which were, after all, not produced to facilitate the measurement we took from them, is questionable.

Additionally, another source of variation could be represented by the provenance of the specimens used for the drawings. In parallel to studies on the gut microbiome of muroid species (e.g., Maurice et al., 2015), intraspecific variation of the stomach ratio might relate to different geographic locations of sub-populations and to seasonal effects, none of which would be controlled in the present study.

A surprising side finding of the present study was the general mismatch between studies that provided quantitative information on the composition of the natural diet, and the quantitative data provided in a large data set on mammal diets (Wilman et al., 2014). The latter data is mainly based on textbook descriptions, assigning the percentage of contribution to the overall diet, based on the sequence in which diet items are listed in these descriptions. Arguably, this procedure may yield information sufficient for broad classifications (e.g., into faunivores, omnivores and herbivores), and congruence with original quantitative data was best for the categories of faunivory/invertivory and herbivory. However, for a diet category we originally considered highly relevant to the present study, that is, granivory, the data congruence was poor (Supporting Information: Table S8). This finding suggests that for more detailed dietary specializations, original literature should be consulted. Nevertheless, in the present study, despite the difference in the data, neither approach confirmed our hypothesis or yielded convincing

dietary patterns. Our observations raise the question to what extent information derived from other large datasets, for example, from the PanTheria database for habitat characteristics, may be subject to similar uncertainties; yet, answering that question was beyond the scope of our study.

The statistical comparison of the stomach ratio with descriptive information on stomach anatomy yielded the self-evident result that stomachs classified as disco-glandular, that is, with a particularly clearly demarcated, small glandular area, have relatively large forestomachs. By contrast, whether the stomachs had a deep angular incision, which often separates the two stomach compartments, explained differences in the stomach ratio to a much lesser degree, and did not add to the fit of the model accounting for the disco-glandular conformation. Apart from these findings, the stomach ratio was not significantly related to any other measure of oral or intestinal anatomy.

A lack of an evident association between the relative forestomach size and diet was among the findings that led Vorontsov (1967) to suggest that adaptation to diet in muroid rodents should be viewed as an interplay of compensating morphologies. This author considered the digestion of plant cell wall to be the main function of the muroid forestomach (for arguments against this interpretation, see Supporting Information: Table S2). He suggested that a large forestomach could compensate for teeth or a large intestine of lesser complexity, and that complex teeth or a complex large intestine could compensate for a smaller forestomach, so that forestomach size itself need not show a relationship with diet. These hypotheses had been illustrated with examples, but not tested statistically. Because of an incongruence of many of his own observations on the muroid hindgut with those of Vorontsov (1967), Behmann (1973) had already challenged this view. In the present study, the absence of any relationship between the stomach ratio and the length of an intestinal component, even when accounting for diet (Supporting Information: Table S5), also speaks against this interpretation. However, due to the low number of species for which stomach and intestine length information was available, and because for both stomach and intestine the state of the material (fresh or conserved) could not be ascertained, this result should not be considered definite.

There is ample evidence that the muroid forestomach mainly harbors a lactic acid-producing microbiome (Supporting Information: Table S2). Many studies found *Lactobacillus* spp. to be the majority of the bacterial flora in the forestomach of muroid rodents (Allan et al., 2018; Kunstýř, 1974; Milovic et al., 2020), leading to a high lactic acid concentration. To our knowledge, the mechanism that selectively maintains this microbiome has not been elucidated, but it can be modulated under laboratory conditions by dietary manipulation (Dubos & Schaedler, 1962). As stated in Section 1, lactic acid-producing bacteria may protect against enteropathogenic microbes, fungi, and fungal mold (Jena et al., 2013; Oliveira et al., 2014). Experimental studies have shown a protective effect of a lactic acid-producing against various toxic and infectious agents (Dubos & Schaedler, 1962). From domestic pigs, it is known that high lactic acid concentrations in the stomach can protect against harmful bacteria

such as *Salmonella* spp. (Canibe et al., 2005; Mikkelsen et al., 2004). Therefore, one could interpret the muroid forestomach as a generic adaptation that is not necessarily diet-related but protects from harmful effects of a variety of biotic influences. Like any other characteristic that sets muroid rodents apart, the forestomach could be hypothesized to be an important contributing factor to their high species diversity and worldwide distribution in numerous habitats. Should one pursue this concept, yet want to take it further to explain the diversity in forestomach morphology, one would have to evoke niches that require particularly high degrees of such protective action, and hence putatively large forestomachs.

Following Shinohara et al. (2016) and Langer and Clauss (2018), we had hypothesized that species with a higher proportion of seeds in their natural diet should have larger forestomachs, as seeds are prone to mold (Janzen, 1976). Additionally, species with food hoarding should have particularly large forestomachs for the same reason; given that seeds are ideal for storage (Vander Wall & Dittel, 2021), granivory and hoarding might show distinct overlap. We had expected niche descriptors that correspond to this scenario (including a distinct habitat seasonality that would foster hoarding behavior) to be statistically linked with the stomach ratio—granivory, cheek pouch presence, food hoarding, latitude, reproductive seasonality, and possibly even habitat humidity. As none of these descriptors showed any relationship with the stomach ratio, our hypothesis must be rejected. This does not contradict the assumption that protection against mold is a major function of the forestomach; this can just not be corroborated using a comparative approach based on forestomach size.

The only dietary factor that was linked to the stomach ratio was invertivory. Various authors suggested that the cornified forestomach might represent a protective adaptation against spiky parts of chitinous exoskeletons or prey that is still alive and putatively biting (Supporting Information: Table S2). As with many other theoretical functions of the muroid forestomach, one counter-argument is that other animals with a similar niche characteristic function without a cornified forestomach—such as shrews or other Eulipotyphla (from the former Insectivora) (Langer, 2017). Given the characteristic gnawing dentition of rodents and their high chewing efficiency (Fritz et al., 2009; Naumova et al., 2021), the assumed presence of live prey and large chitinous particles in their stomach might be dubious and would require detailed documentation. Most notably, other vertebrate predators that are more likely to swallow whole and live prey, like insectivorous reptiles such as chameleons, also function with completely glandular stomachs (Hamdi et al., 2014). Therefore, the adaptive value of a large forestomach for insectivorous species remains unexplained. Given that many muroid species have comparatively large forestomachs that ingest none or only a few invertebrates, the relationship is arguably spurious.

This leaves us with the speculative assumption that the muroid forestomach provides a generic protective function against various challenges, including toxins and infectious diseases linked to high population densities. Using *L. imhausi*, the African crested rat with a particularly large forestomach (Naumova & Zharova, 2003) as a test case, the species is known to live comparatively solitary or in pairs, yet

anoint itself with plant poisons as a defensive strategy (Weinstein et al., 2020). It has been suggested that its large forestomach might be linked to accidental ingestion of these toxins (Kingdon et al., 2012). For this species, the anti-toxin hypothesis would be favored over a high population density hypothesis. In the absence of large comparative datasets on muroid rodent toxin ingestion and social structure, these hypotheses cannot be tested in a comparative manner.

While our data set provides the opportunity for other researchers to test further biological characteristics in comparative approaches, it is evident that an original, large-scale comparative morphological study of the muroid digestive tract would be welcome. In particular, simultaneous quantitative measurements of stomach content composition and dental, stomach, and hindgut morphology in animals of known body mass and size would allow conclusive testing of the theory of compensating adaptations (Vorontsov, 1967). Yet, this may be difficult to put into practice. While different morphological approaches that also address the topography, for example, microCT of intact specimens, or studies recording the gastric peristalsis, more physiological data on enzyme activity, content, and microbiome composition would also be welcome to increase the pool of comparative physiological data, it appears likely that the collection of stomach ratio information will remain the largest comparative data set in terms of sheer species number. A possibly promising approach to elucidate the protective potential of the forestomach microbiome might be experiments that expose muroid rodents to different types of noxious stimuli, including enteropathogenic bacteria, fungi, mold, and other toxins. Arguably, the interest in the function of the muroid forestomach does not justify approaches using surgical excision anymore (DiBattista & Robillard, 1993; Ehle & Warner, 1978; Gärtner & Pfaff, 1979; Kunstýř et al., 1976; Sakaguchi et al., 1981).

Many mammals have adopted ways to use the microbial biomass in their digestive tract as prey—either by the use of coprophagy (Björnhag & Snipes, 1999) or by increasing the harvest of microbes from a forestomach by the use of a washing mechanism (Müller et al., 2011). Muroid rodents appear peculiar in this respect, as they might well use both strategies—coprophagy for the microbes of their own hindgut, and additionally harvesting a protective microbiome from the forestomach. As demonstrated in the present study, a forestomach appears to be a common characteristic of all muroid rodent species. Additionally, the oblique “herringbone-like” internal folds (Supporting Information: Figure S11), providing the colonic separation mechanism that as a prerequisite for the selective retention of microbes and their later ingestion via coprophagy, are also basically common to muroid species (Behmann, 1973; Langer, 2017). It is tempting to ascribe the success of muroid rodents to some degree to this intimate use of their own gastrointestinal microbiome.

5 | CONCLUSION

Similar to the lack of a diet signal in the present study, Alhajeri and Steppan (2018) did not find evidence for an association between diet and diversification rates in muroid rodents and suggested that the

ecological opportunism and flexibility of this group may make a degree of specialization difficult to detect. Evidently, it is a convenient hypothesis to claim that their forestomach makes this group particularly flexible, protecting them from noxious effects of changing to novel foods, novel environments, or high population densities. Evidence for these claims, however, is lacking. Until it is provided, the function of the muroid forestomach remains enigmatic.

AUTHOR CONTRIBUTIONS

Marcus Clauss designed the research. Natalie Steiner and Marcus Clauss performed the literature research on the morphology and function of the muroid forestomach. Marcus Clauss, Louise F. Martin, and Maria J. Duque-Correa dissected the mouse and rat stomachs. Natalie Steiner performed the digitization of literature images and digital measurements. Corina Imper performed the literature research for original quantitative diet information. Carlo Meloro provided the phylogenetic tree for statistical analyses. Maria J. Duque-Correa performed the statistical analyses. Natalie Steiner, Marcus Clauss, and Maria J. Duque-Correa wrote the first draft of the manuscript which later received input from all co-authors.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data are provided as an online supplement to this article.

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