Verifiability of genus-level classification under quantification and parsimony theories: a case study of follicucullid radiolarians

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Abstract.—The classical taxonomy of fossil invertebrates is based on subjective judgments of morphology, which can cause confusion, because there are no codified standards for the classification of genera. Here, we explore the validity of the genus taxonomy of 75 species and morphospecies of the Follicucullidae, a late Paleozoic family of radiolarians, using a new method, Hayashi’s quantification theory II (HQT-II), a general multivariate statistical method for categorical datasets relevant to discriminant analysis. We identify a scheme of 10 genera rather than the currently accepted 3 genera (Follicucullus, Ishigaconus, and Parafollicucullus). As HQT-II cannot incorporate stratigraphic data, a phylogenetic tree of Follicucullidae was reconstructed for 38 species using maximum parsimony. Six lineages emerged, roughly in concordance with the results of HQT-II. Combined with parsimony ancestral state reconstruction, the ancestral group of this family is Haplodiacanthus. Five other groups were discriminated, the Parafollicucullus, Curaibaillella, Pseudoalbaillella, Longtanella, and Follicucullus–Carvier lineages. The morphological evolution of these lineages comprises a minimum essential list of eight states of four traits. HQT-II is a novel discriminant analytical multivariate method that may be of value in other taxonomic problems of paleobiology.

Introduction

Taxonomic classification at the genus level can involve tensions between splitting and lumping philosophies. When there are living taxa, this kind of controversy can often be resolved by molecular data, but it is impossible to use this approach in extinct fossil groups (Sandin et al. 2019).

Radiolarians, ranging in age from the Cambrian to the present, are marine unicellular planktonic rhizarians. We use them here as a test case to explore genus taxonomy, focusing on the family Follicucullidae (Ormiston and Babcock 1979; De Wever et al. 2001). Follicucullids belong to the order Albaillellaria, which is characterized by an internal triangular frame made from three intersecting rods, and species can be defined readily because of their rapid evolution. Albaillellaria are important for stratigraphy, in particular the Follicucullidae, which is the main age-diagnostic clade between the Bashkirian (Pennsylvanian) and the Wuchiapingian (late Lopingian, Permian) (Aitchison et al. 2017).

The family Follicucullidae consists of as many as 75 species, but only three genera (Follicucullus, Ishigaconus, and Parafollicucullus) are regarded as valid, based on the consensus decision of the Paleozoic Genera Working Group.
(Caridroit et al. 2017; Noble et al. 2017). Eleven available genera had been established in this family, but the remaining eight genera were synonymized because of poorly preserved holotypes (e.g., *Longtanella*) or different opinions concerning species-level criteria. Some questions also arose from recently published papers. For example, Nestell and Nestell (2020: p. 10) thought that “*Haploidiacanthus* should be a valid genus” and urged the necessity of reevaluating many follicucullid genera. The poor preservation of the *Longtanella* holotype was agreed by all members without opposition at the time. Later, several Chinese researchers identified some other “true” *Longtanella* species, which impacts on the identity of the toptype of *Longtanella*. It was concluded that this genus differs from *Parafollicucullus* by its turri-form, slightly bent test, and the absence of an obvious wing or pseudothorax (Ito 2020). A further differing opinion concerns *Cariver*: in “The Paleozoic Radiolarian Genera Catalogue,” it is synonymized with *Follicucullus* based on the assumption that differences in the size of the ventral lingula indicate intrageneric variation. However, Nakagawa and Wakita (2020) noted that the developmental location of the ventral lingula is on anatomically opposite sides in both genera. Therefore, it is impossible to explain the differences as intrageneric variation without flipping the anatomical left and right, and thus *Cariver* is identified as a valid genus. Except for this case, however, reasons for identifications of genera have not been clearly explained. Therefore, the subjectivity of these choices to lump or split genera should be tested using more objective means.

Here, we evaluate the traditional genus taxonomy by using two mathematical methods: (1) Hayashi’s quantification theory II (HQT-II), a qualitative discriminant multivariate statistical technique (Dong et al. 1979; Tanaka 1979; Hayashi 1988; Kan and Fujikoshi 2010); and (2) a phylogenetic analysis using maximum parsimony, performed using TNT software. As explained later in detail, the former is a general statistical method to output the correct ratio of predetermined categories whose distinction parameters are based on a qualitative scale. The latter is a method to explore evolutionary relationships. If HQT-II verifies the composition of species based on some genus concept, this could be independent of their evolutionary relationships, whereas the parsimony analysis should provide a robust phylogenetic genus concept that reflects relationships. These two methods are founded on different mathematical backgrounds, so if they converge on the same result, this confirms its robustness. This paper is the first trial of HQT-II and parsimony analysis in radiolarian studies, and this is also probably the first time the method has ever been used in paleontology.

**Material and Methods**

*Meta-dataset.*—The dataset is derived from our own specimens, in particular *Longtanella*, and publications (Supplementary Table 1, Supplementary Fig. 1). The terminology of Follicucullidae species is shown in Figure 1. We adjusted the taxonomic concepts as we have discussed previously (Xiao et al. 2018: p. 199).

The meta-dataset comprises 53 morphological features with 175 states for the 75 taxonomically stable species (including 15 undescribed morphospecies from our own materials). As the applicable measurement scale for both HQT-II and TNT is the statistical ordinal scale or nominal scale of Stevens (1946), morphological features were coded as binary (0, 1) in the case of characters that are present or absent and as a stepped code (0, 1, 2, …) for metric continuous characters (Supplementary Table 2).

*Hayashi’s Quantification Theory II.*—HQT-II is one of four methods of quantification introduced by a Japanese statistician, Chikio Hayashi, who also coined the now widely used term “data science” in 1996. He developed his methods to deal with qualitative data, and they are widely used in Asia in many fields, such as the geologic, environmental, and medical sciences and civil engineering (Hayashi 1950; Matsuba et al. 1998; Li et al. 2005; Takasawa et al. 2010). HQT-II aims at discrimination and classification of samples by establishing discrimination functions based on several variables of known types. It is mathematically equivalent to canonical analysis applied to dummy variables corresponding to categorical data or discriminant analysis in
multidimensional situations. The unique feature of HQT-II and related methods is that all work with qualitative data that can be quantified before analysis using a qualitative external criterion to predict or analyze the effects of the factors while seeking to maximize the correlation ratio (Tanaka 1979).

Among novel methods applied over the years to paleontological questions, numerical taxonomy, introduced to paleobiology in the 1970s, comprises a suite of multivariate statistical methods to handle large databases of numerical and categorical data. As HQT-II was established in the 1950s (e.g., Hayashi 1954), the approach is
older than numerical taxonomy, and we feel it is useful to introduce the method to a wider audience outside Asia. HQT-II represents a third broad statistical approach, different from classic frequentist and Bayesian approaches.

Bayesian statistics have been used ever more widely in recent years, including paleontology (e.g., Xiao et al. 2018), applying algorithms of probability using the likelihood function based on probability theory and random variables. Bayesian discrimination can predict a sample classification based on prior information, but we could not identify any Bayesian methods that are relevant to HQT-II. In comparison to frequentist and Bayesian statistics, HQT-II has the advantage of simplicity. The basic principle of HQT-II is to obtain the centroid of each sample and the center point of each group in multidimensional space and calculate the distance from each sample centroid to the center point. The smallest distance from the centroid of the sample to the center point of the groups determines the group for the sample. This method involves a small amount of calculation and offers high discrimination accuracy associated with weight determination, and is thus suitable for discrimination classification problems that rely on multiple factors.

Before HQT-II analysis, multicollinearity has to be resolved (Kumari 2008), in this case through correlation analysis. We performed HQT-II and associated analyses with the statistical add-on, BellCurve for Excel v. 3.20 (Social Survey Research Information Company). The categorical dataset for HQT-II comprises “categorical external variables” and “classification into more than 2 or 3 groups” (Hayashi 1988; Kan and Fujikoshi 2010). In our study, the former is relevant to assigning species to a genus, whereas the latter is relevant to categorical morphological characters. We performed a correlation analysis, then the HQT-II analysis itself, and then a cluster analysis. HQT-II outputs the following data, including some mathematical requirements: discriminant result (Table 1), correlation ratio \( \eta^2 \) (Table 2), centroid of each group (Supplementary Table 3), range (Table 3, Supplementary Table 4), category score (Supplementary Table 5), sample score (Supplementary Table 6), and group scatter diagram (Fig. 2). The interpretation of these
output data will be explained in the “Results and Interpretations,” where necessary.

The R package RQDA (Huang 2016) can be used to perform qualitative data analyses similar to HQT-II, but as yet there is no full implementation available in R. We provide R code here written by S. Aoki from Gunma University, Japan (Supplement 1 in the Supplementary Material).

**Phylogenetic Analysis.**—Phylogenetic analysis was conducted using the New Technology search in TNT v. 1.5 (Goloboff and Catalano 2016), a standard analytical tool for parsimony analysis. TNT does not evaluate the reliability of assignment of species to genera, but it can output synapomorphy lists at nodes. A time tree was constructed with the paleotree package (Bapst 2012) in R, with stratigraphic ranges of species from their first and last appearance data. We considered only those 38 species with known stratigraphic ranges (Xiao et al. 2018; Zhang et al. 2018), and *Holdsworthella annulata* and *Holdsworthella nodosa* were chosen as outgroup taxa because of their greater stratigraphic age. Then, an ancestral state reconstruction (ASR) of phylogenetically informative characters was conducted at the genus level in R with the ape package (Paradis and Schliep 2019) to track models of trait change.

**Results and Interpretations**

**Correlation Analysis.**—The data matrix size of HQT-II is limited by mathematical requirements and software architecture (Kan 2017). The Bell-Curve for Excel program has a limit of 10 groups for HQT-II. According to the equation (Kan 2017: p. 119), “the number of states minus the number of morphological features” must be reduced to 64. For this purpose, we used Cramer’s V metric in correlation analysis to check for multicollinearity among parameter lists (Supplementary Table 7), and we preferred those morphological features with small absolute values of Cramer’s V (< |0.45|), following the criteria of Kan and Fujikoshi 2010. After several filtering steps (Supplementary Table 8), 25 morphological features were selected for HQT-II (Supplementary Table 9), which is also the objective minimum essential list of morphological characters at the species level that are supported by correlation analysis.

*Hayashi’s Quantification Theory II.*—We first allocated the 75 species to 10 split genera manually, based on comparisons of type species. This a priori classification was evaluated with HQT-II using the 25 morphological characters and 84 morphological states, resulting in 100% discriminant accuracy (Table 1). The discriminant accuracy is a fitness ratio of how well HQT-II predicts the genus to which a species belongs when compared with our manually classified genera. HQT-II outputs the correlation ratio $\eta^2$, the ratio of between-group variation divided by the total variation on each output axis. Referred to $\eta^2$ (0 means no contribution to discriminant result, and 1 is

| Axis 1 | Axis 2 | Axis 3 | Axis 4 | Axis 5 | Axis 6 | Axis 7 |
|--------|--------|--------|--------|--------|--------|--------|
| Correlation ratio $\eta^2$ | 0.9996 | 0.9981 | 0.997  | 0.9894 | 0.9685 | 0.9438 | 0.9287 | 0.8109 | 0.6642 |
FIGURE 2. Group scatter diagram generated from Hayashi’s quantification theory II, showing the visualized display of the clusters of Folliculidae species with two selected axes (six patterns of dimension plots containing: A, axes 1 and 2; B, axes 2 and 3; C, axes 3 and 4; D, axes 4 and 5; E, axes 5 and 6; F, axes 6 and 7).
the full contribution), the first seven axes (>0.9)
make very strong contributions to the discrimi-
nant result (Table 2). According to their
ranges, the characters that correspond to each
axis are objectively calculated (Table 3, Supple-
mentary Table 2). Characters that score high
values (>5.00) give an insight into the combina-
tions of important characters that covary.

The prediction of the sample classification by
HQT-II is determined by the distance of the
sample score from the centroid of each group.
The output of HQT-II is a multidimensional
space of nine axes, and we summarize the data in a two-dimensional scatter diagram (Fig. 2, plotted using the sample score of Supple-
m entary Table 6) with two selected axes
(axes 1 and 2 in the case of Fig. 2A). The origin
(x = 0 and y = 0) is read as the average condition
of all data, the centroid of each genus as the
average condition of the relevant characters
on each axis, and the distances between genera
as the statistical isolation distances. Because the
scores of the first seven axes are so high (>0.9),
all axes look equally important. The maximum
number of combinations of results with two
axes from these seven comprises 21 patterns,
so we cannot show all of them. Instead, six pat-
terns of dimension plots (e.g., axis 1 and 2, axis
2 and 3, ...) are shown in Figure 2. The plot on
axes 1 and 2 (Fig. 2A) shows complete separa-
tion of all 10 genera. The dimension plot
map on axes 2 and 3 (Fig. 2B) shows a continu-
ous group comprising Curvalbaillella, Longta-
nella, Parafollicucullus, and Haplodiacanthus,
which is fit empirically based on our assump-
tions of similarity among these four genera.
The dimension plot map on axes 3 and 4
(Fig. 2C) makes a continuous line between Cur-
valbaillella and Follicucullus, in line with our
empirical observation that they share a similar
very long shell. The plot map on axes 4 and 5
(Fig. 2D) differs from the previous plot maps
in showing two clusters of species: an aggre-
gated generic cluster composed of Follicucullus,
Holdsworthella, Ishigaconus, Kitoconus, and Long-
tanella, and a second cluster of Pseudoalbaillella,
Parafollicucullus, Cariver, and Haplodiacanthus.
These two clusters, however, can also be
thought of as one curve, and if so, this can be
regarded as an example of the “horseshoe or
Guttman effect.” This effect often occurs when
one axis is highly dominant, and the second
axis then becomes a quadratic transformation of the first (Clausen 1998: p. 28). Although
Clausen (1998: p. 28) also commented that
“the horseshoe pattern does not exist here as
an artifact” in some cases, it is presumably the
Guttman effect. The plot map between axes 5
and 6 (Fig. 2E) also looks as if it shows a horse-
shoe pattern if Follicucullus and Cariver are
ignored. The risk of a Guttman effect among
axes 4, 5, and 6 is unclear, but it might well be
suspected. The plot map between axes 6 and
7 (Fig. 2F) forms a curved line except for Curva-
lbaillella and Haplodiacanthus, which might
reflect our feeling that these genera belong to
the same family and the remaining two genera
look different from the majority of Follicuculli-
da. The interpretations of axes are discussed in
the Supplementary Material (item 2) based on
the simple correlation coefficient (Supple-
m entary Table 10).

Cluster Analysis.—The score list output by
HQT-II for the first seven axes was employed
to make a dendrogram for visualization of dis-
tances among the 75 species and morphospecies
using the Ward method (Fig. 3). The dendro-
gram identifies 10 obvious small groups at the
4.0 level, eight midsize groups at the 6.0 level,
and three large groups at the 12.0 level, suggest-
ing that the division into 10 genera is objectively
confirmed. Taking account of the ease of identi-
fication and the hypothesis of the so-called Folli-
cucullus lineages (e.g., Wang et al. 2012; Zhang
et al. 2014), an appropriate number of clusters
is set as eight at a threshold value of 6.0: 17 spe-
cies, all belonging to Longtanella in cluster 1; 4
Curvalbaillella and 2 Kitoconus species in cluster
2, which is synonymized herein as Curvalbai-
lla; 17 Parafollicucullus species in a strict sense
in cluster 3; 5 Haplodiacanthus species in cluster
4; 7 Holdsworthella species in cluster 5; 6 Pseudo-
albaillella species in a strict sense in cluster 6; 3
Follicucullus and 7 Ishigaconus species (synony-
mized herein as Follicucullus) in cluster 7; and
7 Cariver species in cluster 8. It is worth specific
mention that there are no species switches
between genera, as shown by the 100% discrim-
inant ratio under HQT-II.

Phylogenetic Analysis.—After analyzing the
data matrix under the parsimony criterion,
one tree was obtained with tree length 275
Consistency index = 0.400, retention index = 0.701. Most bootstrap values are more than 50. A time-calibrated phylogenetic tree with a geologic timescale and a full stratigraphic range for each taxon is shown in Figure 4. Regardless of the different numbers of species and different mathematical logic between cladistics and HQT-II, the clades in the phylogram coincide with the eight generic clusters discovered by HQT-II, with one exception, indicating the high robustness of the eight-genus division scheme. Figure 4 shows six major clades: (1) Haplodiacanthus (sensu lato)–Holdsworthella (sensu lato)–part of Parafollicucullus clades (lineage I), (2) the remaining Parafollicucullus clade (lineage II), (3) Curvalbaillella (sensu lato) clade (lineage III), (4) Pseudoalbaillella clade (lineage IV), (5) Longtanella clade (lineage V), and (6) Follicucullus–Cariver clades (lineage VI). As shown, Parafollicucullus is a polyphyletic group of lineages I and II. This raises a question about the distinguishing character(s) of Parafollicucullus at the genus level. This extinct polyphyletic group is also considered in terms of homology, because molecular phylogenetic studies for extant Radiolaria and Phaeodaria except for Spumellaria identify strong homology at the level of superfamilies and suborders (Class Acantharea by Decelle et al. [2012], Class Phaeodaria by Nakamura et al. [2015], Order Nassellaria by Sandin et al. [2019], and Order “living Entactinaria” by Nakamura et al. [2020]). The original diagnosis of Parafollicucullus is “bilaterally symmetrical, imperforate siliceous shells of unknown internal structure with apical cone, winged pseudothorax and ring-like pre-pseudoabdominal segment interposed between pseudothorax and pseudoabdomen” (Holdsworth and Jones 1980: p. 285). Parafollicucullus in both lineages I and II possesses these characters, but species in lineage I (Parafollicucullus lomentaria and Parafollicucullus globosa) differ in having a long and straight apical cone, inflated pseudothorax, and undulating pseudoabdomen. These characters are not seen in any species of Parafollicucullus in lineage II. On the other hand, all the species grouped in lineage I (Holdsworthella and Haplodiacanthus) have these three characters. If we simply follow the original diagnosis, “Parafollicucullus” in lineage I is empirically
differentiated from “true” Parafollicucullus, and here we propose Parafollicucullus(?) for the “Parafollicucullus” species in lineage I to distinguish them from Parafollicucullus in lineage II. These characters were overlooked before. This morphological parameter appeared with high scores (>5.00) on axes 4, 6, and 7 in HQT-II (Table 2). The principle in HQT-II is mathematical independence among the axes, but several similar morphological parameters were scattered on different axes in HQT-II. This might be helpful to determine the cause of mismatch between both methods.

Ancestral State Reconstructions.—The characters evaluated for ASR under the equal-rates model are those that have larger values in the range output by HQT-II: curvature (character 1), height (character 2), and size (character 3) of the apical cone, extent (character 4) and shape (character 5) of the flaps, bands (character 6) and segmentations (character 7) of the pseudoabdomen, and inflation of the pseudothorax (character 8). It turns out that most descendants kept the plesiomorphic state for almost all characters (Fig. 5), and the distributions of some characters represent clear generic-level groupings. We trace morphological changes across the phylogeny in chronological order.

As shown by green and blue dots in characters 1, 2, and 3 (Fig. 5, color figure online), the
apical cone of the ancestral species is straight, and medium in height and size. Most descendants inherit the straight apical cone, except for lineage IV, where it is strongly curved, and lineage II, where it is slightly curved. The height and size of the apical cone varies greatly in the descendants, while lineage VI obviously possesses a larger apical cone than the others (red dots in character 2 and gray dots in character 3). By contrast, the apical cone of lineages II and III became smaller (blue dots in character 2 and character 3). The flaps of the ancestral species are blade-like and extend obliquely downward, and most descendants kept this feature, while members of the 

Figure 5. Parsimony ancestral state reconstruction based on the phylogenetic tree for the eight chosen taxonomically important morphological characters in a maximum likelihood framework. Different states of traits are colored. Pie charts represent empirical Bayesian posterior probabilities (trait values) of ancestral states for each node in the phylogenetic tree. See caption for Fig. 3 for genus abbreviations. PA, pseudoabdomen; PT, pseudothorax.

Overall, for all lineages, plesiomorphies are a straight apical cone with medium height and size, blade-like flaps that extend obliquely downward, three-segmented pseudoabdomen without bands, and uninflated pseudothorax (Table 4, Fig. 6). Synapomorphies for different lineages include short apical cone in lineage II, single segmentation of pseudoabdomen in lineages III and IV, strongly curved apical cone in lineage IV, and large apical cone more than \( \frac{1}{2} \) of the shell height in lineage VI.

Discussion

Taxonomic Concepts and Data Manipulation.— In coding character states, we found that
holotypes are generally the best-preserved specimens of the wider sample published with the initial descriptions. Therefore, in our analysis, we largely refer to holotypes, but checked the accuracy of character coding with paratypes. In rare cases, we used paratypes if the holotype specimen was partly broken. Species variation was considered in compiling the synonymy lists and the illustrations (e.g., Wang et al. 2012; Ito et al. 2015) and our own tests. A categorical approach was used to deal with species variation. If a character has extensive variation, two or more character states were assigned to some species (see Supplementary Table 2). In some cases, we coded character states as “?” if there was no information in the literature.

Further, we took care in designing intervals of continuous quantitative characters. Different specimens within a single species may vary in some characters, for example, the height of the pseudothorax. Thus, when the characters were designed, we used the widest intervals of length ratios to accommodate the maximum number of specimens within a species, like $\frac{1}{2} - \frac{3}{4}$.

An Objective Method to Choose Distinguishing Morphological Features at the Genus Level.—The motivation of this study was to evaluate the plausibility of genus definitions based on splitting and lumping. The prior three-genera scheme for the Follicucullidae (Parafollicucullus, Ishigaconus, Follicucullus) was based on the quality of the type material and descriptions that rely on distinct characters. In the case presented here, six lineages are favored, with one lineage lumping two of the three genera from the Paleozoic catalogue (Ishigaconus + Follicucullus) and some previously poorly described
and figured genera, especially *Longtanella*, being reconstituted based on a wider character matrix across all species within the clade than was used in the original typological work.

Correlation analysis was applied first to reduce any multicollinearity, in case related characters might artificially dominate the results. For example, the apical cone angle largely decides the apical cone size of radiolarians, as shown by their high correlation coefficient. This could be useful for detecting more inconspicuous related characters in other biota. In the current case study, HQT-II has proved to be a powerful tool to evaluate genus categories. The advantage of HQT-II is its reliability in a wide variety of scientific applications as a general multivariate analysis method, and it is applicable for any species whose stratigraphic ranges are unknown. Compared with discriminant analysis, whose explanatory variables can only be quantitative, HQT-II is more flexible in dealing with classification in paleontology. Qualitative and quantitative characters of taxa can be readily coded and processed without weight determination.

**Morphological Evolution of Six Lineages and Competing Models.**—HQT-II and cladistics resulted in a clear demonstration of the taxonomic validity of the genera *Haplodiacanthus* (lineage I), *Parafollicucullus* (lineage II), *Curvalbaillella* (lineage III), *Pseudoabaillella* (lineage IV), *Longtanella* (lineage V), and *Follicucullus* and *Cariver* (lineage VI). These objective lineage trees permit us to reconstruct morphological evolution and evaluate the likelihood of previous evolutionary studies. Our attempt to specify morphological characters at the genus level produced a minimal list of eight possible conditions of four traits (curvature, height, and size of apical cone; extension pattern and shape of flaps; inflated condition of pseudothorax; number of bands and segments in pseudoabdomen) (Table 5). These eight characters were evaluated through ASR, because they scored high values in the range output by HQT-II, meaning they are the most important in defining the different groups. Table 5 and Figure 5 also show that it is very difficult to pinpoint a genus name by a single state of a single trait; a combination is essential. In reference to the original definition/diagnosis for each genus, a huge number of morphological characters can be excluded from the minimum essential list (the rightmost column of Table 5). As listed in Table 5, 88 cells comprising eight morphological states and 11 “genera” are filled with state conditions. By comparison with the original description of each genus, 9 of the 88 cells (10.2%) exactly match the original description. Eleven of the 88 cells (12.5%) partly match the original description. The remaining 68 of the 88 cells (77.3%) are not clearly written in the original description. As Noble et al. (2017) suggested, this means it is essential to revise the genus definitions, but some problems remain in the minimum essential character list, as discussed later.

The dated phylogeny (Fig. 4) and the ACS trees (Fig. 5) can be compared in a general way. For example, we include in Figure 4 only species whose time range is known, so some taxa from Figure 5 are not included. Noting that we show the known fossil ranges in Figure 4 (thick black lines) as well as the minimum inferred ranges (thin black lines), we can compare the major lineages identified through the HQT-II method (Fig. 5).

Lineage I is the *Haplodiacanthus* (sensu lato)—*Holdsworthella* (sensu lato)—*Parafollicucullus* (?) clades. In this lineage, species possess a medium-sized apical cone and multiply segmented pseudoabdomen. Lineage I ranges from the Gzhelian to middle Capitanian, but diverges from other lineages in the early Moscovian. The previously identified portion of lineage I was limited to “*Pa. longtanensis-Pa. globosa*” by Wang et al. (2012). This lineage is marked by decreasing segmentation of the pseudoabdomen (Fig. 7), supporting the importance of this character. Those *Parafollicucullus* (?) species that fall in lineage I (*Pa.* (?) *lomentaria, Pa.* (?) *globosa, Pa.* (?) *longtanensis*) may be placed in a new genus in future in order to resolve the polyphyletic condition.

Lineage II is the *Parafollicucullus* clade exclusive of lineage I *Parafollicucullus* (?). All species in this lineage possess a short and slightly curved apical cone. It is noted that the type species of *Pseudoabaillella* (*Ps. scalprata*) is placed in lineage IV after node 3, where lineage II (*Curvalbaillella* sensu lato) diverges from lineages III–VI (Fig. 6). As far as we know, there has been no
| Lineage | Traditional taxonomy | Suggested genus taxonomy | Curvature | Height | Size | Extension patterns | Shape | Inflation | Band | Segmentation | Abandoned morphological characters |
|---------|----------------------|--------------------------|-----------|-------|------|-------------------|-------|-----------|------|--------------|----------------------------------|
| **Lineage I** | *Pseudoalbaillella* | *Parafollicucullus* (?)(Lineage I) | Striaghtly curved | Medium | Medium | Vertically or obliquely downward | Blade-like | Slightly or strongly inflated | Absent | Two or three | Bilaterally symmetrical, shell imperforate, ring-like post-pseudothorax waist present |
|         |                      | *Holdsworthella* | Slightly curved | Medium | Medium | Obliquely downward | Blade-like | Slightly inflated | Absent | Three or four | Apical cone segmented, pseudothorax with two strong spines, columellae robust with two pores on the distal part |
|         |                      | *Haplodiacanthus* | Straight, rarely slightly curved | Medium or 1/2 of the shell height | Medium | Horizontal, vertically or obliquely downward | Blade-like | Slightly inflated, rarely not inflated | Absent, or three to four | One or three | Shell imperforate, lamellar, columellae elongated parallel to the shell wall, apical cone segmented, distal part curved |
| **Lineage II** |                      | *Parafollicucullus* (Lineage II) | Slightly curved | Small or medium | Small or medium | Horizontal and obliquely downward | Blade-like | Not inflated | Absent, rarely five | Two, rarely one or four | Bilaterally symmetrical, shell imperforate, ring-like post-pseudothorax waist present |
|         |                      | *Curvalbaillella* | Straight, very rarely curved | Short | Small | Horizontal or vertically and obliquely upward | Blade-like | Not inflated | Absent, rarely more than five | One | Test imperforate, apical cone unsegmented; pseudothorax small; pseudoabdomen long; entirely curved in distal part; columellae free distally; aperture large, straight, and oval |
|         |                      | *Kitocomus* | Straight or slightly curved | Short or 1/4 of the shell height | Medium | Vertically or obliquely downward | Blade-like | Not inflated | Absent | One | Shell imperforate, apical cone unsegmented, pseudoabdomen cylindrical and very long, distal part slightly bent ventrally |
| **Lineage IV** |                      | *Pseudoalbaillella* | Strongly curved, rarely slightly curved | Medium to Medium >1/2 of the shell height | Medium | Obliquely downward | Blade-like | Slightly to strongly inflated | Absent | One | Bilaterally symmetrical, shell imperforate |
| Lineage | Traditional taxonomy | Suggested genus taxonomy | Apical cone | Flaps | Pseudothorax | Pseudoabdomen | Abandoned morphological characters |
|---------|----------------------|--------------------------|-------------|-------|--------------|--------------|-----------------------------------|
| Lineage V | | | Longtanella | Straight | Very short to $\frac{1}{2}$ of the shell height | Very small to large | Vertically or obliquely downward | Blade-like | Not inflated to strongly inflated | Absent | One to four | Shell smooth and straight, bilaterally symmetrical turritiformis, shell divided into the spire, turri-body, and turri-bottom; four flaps |
| Lineage VI | Folliculhus Ishigaconus | | Straight or “U” shaped | $>\frac{1}{4}$ of the shell height | Large | Obliquely downward | Blade-like | Not inflated | Absent | Absent, rarely one | Test imperforate and very slender, no wings, aperture large, free columellae proximally connected, part of the distal part slightly curved |
| Folliculhus | Straight | $>\frac{1}{4}$ of the shell height | Large | Obliquely downward | Blade-like | Slightly inflated | Absent | One | Shell imperforate, aperture elliptical and skirt-like, longitudinal ribs join the apex of the shell |
| Cariver | Straight or slightly curved | $>\frac{1}{2}$ of the shell height | Large | upward or downward | Massive, rarely blade-like | Moderately inflated | Absent | One | Apical cone unsegmented, pseudothorax large, post-pseudothorax waist ventralward curved, aperture oval, sinus present |
previous phylogeny connecting the type species of Pseudoalbaillella and Parafollicucullus (Pa. fusi-
formis). Instead, Wang et al. (2012) recognized
two lineages “Pa. ishigai-Pa. longtanensis-Pa. fusi-
formis” and “Pa. fusiformis-Pa. internata-Pa. mona-
cantha” (Fig. 7). Our result excludes Pa. longtanensis from lineage I. The first lineage
involves transitions in decrease of segmentation of pseudoabdomen, whereas the second involves
transitions in degeneration of the ventral wing. Although the latter character is not recognized
in this study as an important trait in genus-level groupings, the phylogram (Fig. 4) supports
their opinions, except for Pa. longtanensis.

Lineage III is the Curvalbaillella (sensu lato)
clade, and we include here members of the
genus Kitoconus, which we identify also as Cur-
valbaillella. The difference between these two
genera is the curved or straight long pseudoab-
domen. Our result is that species in lineage III
have a straight apical cone, an uninflated pseu-
doctorax, and a long unsegmented pseudoab-
domen that is distinct from other taxa. We
found no necessity to separate Curvalbaillella
and Kitoconus, but morphotypes with the typ-
ical curved pseudoabdomen are limited to
between the latest Gzhelian and early Sakmar-
ian, so that an artificial division between the
two genera may be allowable.

Lineage IV is the Pseudoalbaillella (sensu stricto) clade, in which the species possess a
curved and higher apical cone and unseg-
mented pseudoabdomen. The verified range of Pseudoalbaillella (sensu stricto) is from late
Asselian to latest Roadian. Ishiga (1983) pro-
posed that Pseudoalbaillella evolved without
Parafollicucullus (sensu stricto), and he thought
that Ps. scalprata gave rise to Ps. postscalprata,
which in turn gave rise to Ps. rhombothoracata
(Fig. 7). Our phylogram partly supports this
idea that some of the sister taxa may have direct
evolutionary relationships.

Lineage V is the Longtanella clade. The species
in lineage V have a straight apical cone without
(or with weakly developed) wings. The evolu-
tionary position of lineage V is involved in the
evolutionary relationship among Parafollicucul-
lus, Pseudoalbaillella, and Folliculus in that

Figure 7. Prior models on the “Pseudoalbaillella” lineages recognized in Follicucullidae from previous work (Ishiga 1983; Wang et al. 2012) and our models. Abbreviations: Kas., Kasimovian; Gzh., Gzhelian; Ass., Asselian; Sak., Sakmarian; Roa., Roadian; Wor., Wordian; Cap., Capitanian.
Longtanella has been ignored in previous evolutionary models. Follicucullus was thought to originate from Pseudoalbaillella, because Pa. monacantha used to be considered a species of Follicucullus (Ishiga 1991; De Wever et al. 2001; Zhang et al. 2014). However, Wang et al. (2012) noticed that it is better to place Pa. monacantha in Pseudoalbaillella because of the evolutionary transitions, as mentioned earlier, and this opinion was confirmed by Ito et al. (2015). Ito et al. (2016) also drew a direct evolutionary connection from Parafollicucullus to Follicucullus, but this was not supported by our analyses, in which we identify a relationship between Longtanella and Follicucullus (Fig. 4).

Lineage VI is the Follicucullus–Cariver clade. The species belonging in this lineage are unwinged conical types with large apical cones, species of Cariver and Follicucullus. Noble et al. (2017) synonymized Cariver with Follicucullus, but they clearly form distinct clades within lineage VI (Fig. 4). Noble et al. (2017: p. 427) gave their reasons as “the type species falls well within the original diagnosis,” but this decision is rejected, because the flap develops on anatomically opposite sides in both genera (Nakagawa and Wakita 2020).

Some further lineages were recognized, such as F. scholasticus-F. bipartitus-F. hamatus, which developed by increasing the curvature of the apical cone, and F. ventricosus-Ca. guangxiensis-Ca. charveti-Ca. orthogonus which developed by changing the direction of the flaps and the inflation of the pseudothorax (Caridroit and De Wever 1986; Wang et al. 2012; Zhang et al. 2014; Fig. 8). It should be noted that these key transitions are consistent with our eight morphologically important characters.

 Unsolved Issue.—This paper has focused on a reevaluation of the genera in the family Follicculidae using a range of objective computational methods, namely HQT-II, TNT, paleotree, and ASR. Using multivariate statistical procedures, morphological characters were selected to avoid multicollinearity. The minimum required number of distinguishing characters was limited to eight parameters (curvature, height, and size of apical cone; extension patterns and shape of flaps; inflated condition of pseudothorax; number of bands and number of segments in pseudoabdomen) (Table 5). This contributes to lowering the burden of observation with many morphological characters. These morphological characters, however, may be lost or unseen in poorly...
preserved follicucullid specimens. However, specialists generally seem to correctly specify the genus. This phenomenon may be understood if we consider the morphological characters omitted in the process of checking multicollinearity (Supplementary Table 7). For example, if the curvature of the apical cone cannot be examined in real samples, morphological characters with high correlation coefficient values, such as orientation of the apical cone ($r = 0.87$) (“Apical cone_Orientation” in Supplementary Table 7) and composition of overall shell ($r = 0.64$) (“Overall shell_consist” in Supplementary Table 7) may be used as alternative morphological characters. It looks strange to use “composition of overall shell,” but it may be allowable if we accept the value of the correlation coefficient. For practical identification, such alternative morphological characters become part of the definition of the genus. Morphological characters that are omitted in the process of reducing multicollinearity can be used as a backup, reflecting their statistical “redundancy,” for practical identification of poorly preserved specimens. Such “redundancy” is not achieved by the simple addition of “omitted morphological characters” in the diagnosis, because these cannot be objectively tested. We hope to consider the redundancy issue further in future.

**Conclusions**

The first application of HQT-II, TNT, paleotree, and ASR to the Permain follicucullid radiolarians has tested the current three-genera scheme, their hypothesized evolutionary history, and morphological evolution for eight selected morphological characters. The combination of HQT-II and parsimony analysis showed that the three-genera scheme with *Follicucullus, Ishigaconus,* and *Parafollicucullus* cannot be sustained, and that instead the family should be subdivided into 10 genera consisting of 17 *Longtanella* species, 17 *Parafollicucullus* species, 6 *Pseudoalbaillella* species, 6 *Curvalbaillella*-species, 12 *Haplodiacanthus* species, 10 *Follicucullus* species, and 7 *Cariver* species. The discrimination of this genus-level solution was supported 100% by the HQT-II analysis.

The phylogenetic tree analysis objectively output six follicucullid lineages. *Parafollicucullus* used to be thought of as the direct ancestor of *Follicucullus*. The validity of *Longtanella* has been suspected for decades, but this genus is an important sister group in evolution between *Pseudoalbaillella* and *Follicucullus*. *Pseudoalbaillella, Longtanella, Follicucullus,* and *Cariver* shared a recent common ancestor, while *Parafollicucullus* is polyphyletic and not so closely related to them.

Eight characters (curvature, height, and size of apical cone; extension patterns and shape of flaps; inflated condition of pseudoabdomen) were chosen for their potential to discriminate species at the genus level based on their larger values in the range output by HQT-II. Then we challenged these eight traits in their ability to discriminate six lineages, and it turned out that the key transitions recognized in prior models are consistent with these eight morphologically important characters. Moreover, the usability of these important morphological characters may help to lower the burden of observation on many traits, especially for poorly preserved specimens.

Finally, we proposed a protocol to discriminate a genus-level divisional scheme and reconstruct the phylogeny: (1) preparation of a categorical dataset for each species in a family; (2) reduction of multicollinearity with correlation analysis; (3) evaluation of the current genus scheme with HQT-II; (4) confirmation of genus divisions by a cluster analysis with ranges from HQT-II; (5) reconstruction of phylogenetic trees with stratigraphically documented species from the full set of species from HQT-II; and (6) determination of major morphological characters in evolution. This protocol is functional, as shown by the case study of Permain radiolarians, and it can be applied to other taxa of macro- and microfossils in the whole field of paleontology.

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Holdsworth, B. K., and D. L. Jones. 1980. Preliminary radiolarian
Hayashi, C. 1988. New developments in multidimensional data
Ishiga, H. 1983. Morphological change in the Permian Radiolaria,
Pseudoalbaillella scalprata in Japan. Transactions and Proceedings of the Palaeontological Society of Japan 129:1–8.
Ishiga, H. 1991. Description of a new Follicicullus species from southwest Japan. Memoirs of the Faculty of Science, Shimane University 25:107–118.
Ito, T. 2020. Taxonomic re-evaluation of the Permian radiolarian genus Longanella Sheng and Wang (Follicicullidae, Albaellaria). Revue de Micropaléontologie 66:100406.
Ito, T., Q. L. Feng, and A. Matsuoka. 2015. Taxonomic significance of short forms of middle Permian Pseudoalbaillella Holdsworth and Jones, 1980 (Follicicullidae, Radiolaria). Revue de Micropaléontologie 58:3–12.
Ito, T., Q. L. Feng, and A. Matsuoka. 2016. Possible boundaries between Pseudoalbaillella and Follicicullus (Follicicullidae, Albaellaria, Radiolaria): an example of morphological information from fossils and its use in taxononmy. Forma 31:7–10.
Kan, T. 2017. Training for multivariate analysis with examples and exercises on Excel—survival analysis, logistic analysis and time series analysis. Ohm-sha, Tokyo. [In Japanese.]
Kan, T., and Y. Fujikoshi. 2010. Qualitative method type II—a discriminant analysis for qualitative data. Gennai-Sugakusha, Tokyo. [In Japanese.]
Kumari, S. S. S. 2008. Multicollinearity: estimation and elimination. Journal of Contemporary Research in Management 3:87–95.
Li, N., W. Gu, N. Okada, and J. K. Levy. 2005. The utility of Hayashi’s quantification theory for assessment of land surface indices in influence of dust storms: a case study in Inner Mongolia, China. Atmospheric Environment 39:119–126.
Matsuba, T., C. R. Ding, L. Liu, and Y. Chiba. 1998. The utility of Hayashi’s quantification theory type II for the rapid assessment of the epidemiological survey in the developing countries—in a case of the vaccine coverage survey in Yunnan Province, China. Journal of Epidemiology 8:24–27.
Nakagawa, T., and K. Wakita. 2020. Morphological insights from extremely well-preserved Panfollicicillus (Radiolaria, Order Albaellaria) from the probable Roadian (Guadalupian, middle Permian) manganese nodule in the Nishiki Group of the Akiyoshi Belt, southwest Japan. Palaeontological Research 24:161–167.
Nakamura, Y., I. Imai, A. Yamaguchi, A. Tuji, and N. Suzuki. 2015. Molecular phylogeny of the widely distributed marine protists, Phaeodaria (Rhizaria, Cercozoa). Protist 166:363–373.
Nakamura, N., M. M. Sandin, N. Suzuki, A. Tuji, and F. Not. 2020. Phylogenetic revision of the Order Entactinaria—Paleozoic relict Radiolaria (Rhizaria, SAR). Protists 127:125712.
Nestell, G. P., and M. K. Nestell. 2020. Roadian (earliest Guadalupian, Middle Permian) radiolarians from the Guadalupe Mountains, west Texas, USA. Part I: Albaellaria and Entactinaria. Micropaleontology 66:1–50.
Noble, P., J. C. Aitchison, T. Danielian, P. Dumitruc, J. Maletz, N. Suzuki, J. Cuvelier, M. Caridroit, and L. O’Dogherty. 2017. Taxonomy of Paleozoic radiolarian genera. Geodiversitas 39:419–502.
Ormiston, A., and L. Babcock. 1979. Follicicillus, new radiolarian genus from the Guadalupian (Permian) Lamar Limestone of the Delaware Basin. Journal of Paleontology 53:328–334.
Paradis, E., and K. Schliep. 2019. ape 5.0: an environment for modellineg molecular classiﬁcation of Nassellaria (Radiolaria). Protist 170:187–208.
Stevens, S. S. 1946. On the theory of scales of measurement. Science 103:877–880.
Takasawa, T., M. Tanaka, Y. Gonda, and H. Kawabe. 2010. Characteristic analysis of landslides and slope failure in the Imo River

Literature Cited
Aitchison, J. C., N. Suzuki, M. Caridroit, T. Danielian, and P. Noble. 2017. Paleozoic radiolarian biostatigraphy. Geodiversitas 39:503–531.
Bapst, D. W. 2012. paleotree: an R package for paleontological and phylogenetic analyses of evolution. Methods in Ecology and Evolution 3:803–807.
Caridroit, M., and P. De Wever. 1986. Some Late Permian radiolarians from pelitic rocks of the Tatsuno Formation (Hyogo Prefecture), southwest Japan. Marine Micropaleontology 11:55–90.
Caridroit, M., T. Danielian, L. O’Dogherty, J. Cuvelier, J. C. Aitchison, L. Pouille, P. Noble, P. Dumitruc, N. Suzuki, K. Kuwahara, J. Maletz, and Q. L. Feng. 2017. An illustrated catalogue and revised classification of Paleozoic radiolarian genera. Geodiversitas 39:363–417.
Clausen, S. E. 1998. Applied correspondence analysis. An introduction. Sage, London.
Decolle, J., N. Suzuki, F. Mahé, C. de Vargas, and F. Not. 2012. Molecular phylogeny and morphological evolution of the Acantharia (Radiolaria). Protist 163:435–450.
De Wever, P., P. Dumitruc, J. P. Caulet, C. Nigrini, and M. Caridroit. 2001. Radiolarians in the sedimentary record. Gordon & Breach, Amsterdam.
Dong, W. Q., G. Y. Zhou, and L. X. Xia. 1979. Quantitative theory and its application. Jilin People’s Publishing House, China. [In Chinese.]
Goloboff, P. A., and S. A. Catalano. 2016. TNT version 1.5, including a full implementation of phylogenetic morphometrics. Cladistics 32:221–238.
Hayashi, C. 1950. On the quantification of qualitative data from the mathematically-statistical point of view (an approach for applying this method to the paleo prediction. Annals of the Institute of Statistical Mathematics 3:35–47.
Hayashi, C. 1954. Multidimensional quantification. II. Proceedings of the Japan Academy 30:165–169.
Hayashi, C. 1988. New developments in multidimensional data analysis. Pp. 3–16 in C. Hayashi, ed. Recent development in clustering and data analysis. Academic Press, Boston, Mass.
Holdsworth, B. K., and D. L. Jones. 1980. Preliminary radiolarian zonation for late Devonian through Permian time. Geology 8:281–285.
Huang, R. G. 2016. RQDA: R-based qualitative data analysis, R package version 0.2-8. http://rqi.a.r-forge.r-project.org, accessed 22 June 2020.
Ishiga, H. 1983. Morphological change in the Permian Radiolaria, Pseudoalbaillella scalprata in Japan. Transactions and Proceedings of the Palaeontological Society of Japan 129:1–8.
Basin induced by the mid Niigata Earthquake using GIS. Pp. 632–641 in Interpraevent 2010 Symposium Proceedings. International Research Society, Taipei.

Tanaka, Y. 1979. Review of the methods of quantification. Environmental Health Perspectives 32:113–123.

Wang, Y. J., H. Luo, and Q. Yang. 2012. Late Paleozoic radiolarians in the Qinfang area, southeast Guangxi. China University Science Technical Press, Hefei.

Xiao, Y. F., N. Suzuki, and W. H. He. 2018. Low-latitudinal standard Permian radiolarian biostratigraphy for multiple purposes with unitary association, graphic correlation, and Bayesian inference methods. Earth-Science Reviews 179:168–206.

Zhang, L., T. Ito, Q. L. Feng, M. Caridroit, and T. Danelian. 2014. Phylogenetic model of Follicucullus-lineages (Albaillellaria, Radiolaria) based on high resolution biostratigraphy of the Permian Bancheng Formation, Guangxi, South China. Journal of Micropalaentology 33:179–192.

Zhang, L., Q. L. Feng, and W. H. He. 2018. Permian radiolarian biostratigraphy. Geological Society of London Special Publication 450:143–163.