

POSTILLA

Published from 1950 to 2004, the short papers of the *Postilla* series reported on original research by the Yale Peabody Museum of Natural History's curators, staff, and research associates, and their colleagues, in the natural science disciplines represented by the collections of the Museum's curatorial divisions.

The *Postilla* series, which ceased publication with Number 232 (2004), was incorporated into the journal *Bulletin of the Peabody Museum of Natural History*, available from BioOne Complete at <https://bioone.org/>.

Yale Peabody Museum scholarly publications are archived through EliScholar, a digital platform for scholarly publishing provided by Yale University Library at <https://elischolar.library.yale.edu/>.



This work is licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 4.0 International License.
<https://creativecommons.org/licenses/by-nc-sa/4.0/>

Yale PEABODY MUSEUM OF NATURAL HISTORY

P.O. Box 208118 | New Haven CT 06520-8118 USA | peabody.yale.edu

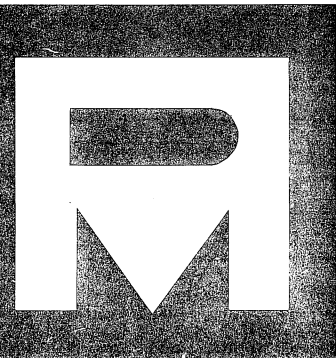
POSTILLA
PEABODY MUSEUM
YALE UNIVERSITY

NUMBER 146

18 AUGUST 1970

MORPHOLOGICAL VARIATION
IN GAMMARUS MINUS SAY
(AMPHIPODA, GAMMARIDAE),
WITH EMPHASIS ON
SUBTERRANEAN FORMS

JOHN R. HOLSINGER
DAVID C. CULVER





POSTILLA

Published by the Peabody Museum of Natural History, Yale University

Postilla includes results of original research on systematic, evolutionary, morphological, and ecological biology, including paleontology. Syntheses and other theoretical papers based on research are also welcomed. *Postilla* is intended primarily for papers by the staff of the Peabody Museum or on research using material in this Museum.

Editors: Nancy A. Ahlstrom, Elizabeth G. Weinman, Elise K. Kenney.

Postilla is published at frequent but irregular intervals. Manuscripts, orders for publications, and all correspondence concerning publications should be directed to:

**Publications Office
Peabody Museum of Natural History
New Haven, Conn., 06520, U.S.A.**

Lists of the publications of the Museum are available from the above office. These include *Postilla*, *Bulletin*, *Discovery*, special publications, and available back numbers of the discontinued journal, *Bulletin of the Bingham Oceanographic Collection*. All except *Discovery* are available in exchange for relevant publications of other scientific institutions anywhere in the world.

MORPHOLOGICAL VARIATION IN GAMMARUS MINUS SAY (AMPHIPODA, GAMMARIDAE), WITH EMPHASIS ON SUBTERRANEAN FORMS

JOHN R. HOLSINGER

Department of Biology, Old Dominion University,
Norfolk, Virginia 23508

DAVID C. CULVER

Department of Biology, University of Chicago,
Chicago, Illinois 60637

(Received November 26, 1969)

ABSTRACT

Gammarus minus Say is a common amphipod species in springs and caves of limestone areas of the eastern and middle-eastern United States. Samples of populations from the central Appalachians were examined closely and morphological variation between spring and cave populations was analyzed. This species occurs in three morphological forms: a spring form, an intermediate cave form and an extreme cave form. The latter form was termed variety *tenuipes* by some earlier workers but has no nomenclatural validity. In contrast to the spring form, the cave forms show a reduction in eye structure, a change in pigmentation of the integument and a proportionate increase in the length of some of the appendages. It is concluded that *G. minus* is an extremely vagile and highly variable species that can occupy a variety of habitats, ranging from surface springs to small or large cave systems in certain karst areas.

INTRODUCTION

Gammarus minus Say is a common inhabitant of springs, small spring-fed streams and cave streams in the eastern United States but is most often encountered in springs developed in limestone areas. This species was originally described by Say (1818), but subsequent references to it were few. Shoemaker (1940) carefully redescribed *G. minus* and gave a thorough literature review of the species up until that time. In the same treatment, Shoemaker (1940) synonymized *G. propinquus* Hay and *G. purpurascens* Hay with *G. minus* and designated a variety which he called *tenuipes*. Hubricht (1943) added numerous new locality records for this species and discussed the variety *tenuipes*. The species was again treated systematically by Bousfield (1958), who confirmed the earlier synonymies of Shoemaker and suggested that the variety *tenuipes* might be a distinct troglobitic (obligatory cavernicole) species. More recently, Minckley and Cole (1963) analyzed the morphological variation in several populations of *G. minus* from spring-fed streams in north-central Kentucky and compared the ecology and morphology of these populations with sympatric populations of *G. bousfieldi* Cole and Minckley. Finally, this species was compared biogeographically with some other members of the family Gammaridae in a paper on the biogeography of freshwater amphipods of the southern and central Appalachians by Holsinger (1969).

Both Shoemaker (1940) and Hubricht (1943) treated the morphological variation in *G. minus* as it related to cave populations in terms of "varieties." Presumably, Shoemaker and Hubricht used the term "variety" to signify a unified morphological group that apparently had become restricted to subterranean waters. Whether these workers were using the variety *tenuipes* to express genetic difference or to express the extreme variation of a plastic phenotype, or both, cannot be determined. Nevertheless, Shoemaker (1940) formally designated several cave populations of *G. minus* from Greenbrier County, West Virginia as a taxonomic variety under the name *tenuipes*. This name was later used by Hubricht (1943) and Bousfield (1958), but not necessarily in a nomenclatural sense.

Although Shoemaker described *tenuipes* as a "variety," he designated a type and created a trinomen. However, it was not clear whether he intended this taxon to be a subspecies or an infra-

subspecific group. *The International Code of Zoological Nomenclature* (Stoll et al., 1964, Article 45) allows a certain amount of freedom in dealing with "varieties" and "forms" proposed prior to 1961. Therefore, based on our interpretation of the Code, the variety *tenuipes* is considered to be of infrasubspecific rank and should not be accorded taxonomic validity.

While Shoemaker (1940) recognized a single variety (*tenuipes*) of *G. minus*, Hubricht (1943) went further and divided the species into three varieties or forms, but he did not treat them in a nomenclatural manner. The three varieties recognized by Hubricht were: a brown spring form with well-developed eyes and short antennae; a bluish intermediate cave form with slightly reduced eyes and long antennae; and a bluish fragile-bodied cave form with greatly reduced eyes and long antennae (i.e., *tenuipes*).

To facilitate discussion in this paper, three morphological groups, approximately equal to those described by Hubricht (1943), will be assigned Roman numerals and designated as follows: "*tenuipes*" Form I, intermediate cave Form II, and spring Form III.

The objectives of this study were: 1) to quantify and describe the morphological variation in central Appalachian populations of *G. minus* with particular reference to subterranean populations; 2) to clarify the morphological status of the three forms of *G. minus*; and 3) to demonstrate that the subterranean "variety" *tenuipes* is an ecophenotype or extreme morphological variant of the more common and widespread spring form.

During the course of this study we critically examined 222 collections of *G. minus* from 179 different localities in the Appalachian region. A breakdown of the locality data follows: three caves, four springs and one spring-run in three counties of northern Alabama; one spring in one county of northwestern Georgia; seven springs and two spring-runs in five counties of southern Indiana; three caves and five springs in five counties of central and eastern Kentucky; two caves, five springs and one spring-run in three counties of central Maryland; five caves, nine springs and three spring-runs in eight counties of central and southern Pennsylvania; three caves, six springs and seven spring-runs in nine counties of central and eastern Tennessee; nine caves, 30 springs and one spring-run in 16 counties of northern and western Virginia; 46 caves, 24 springs and two spring-runs in eight counties in eastern West Virginia. In addition to the above, samples were examined in less detail of *G. minus* from

40 localities in southern Indiana, Kentucky, southern Ohio and central Tennessee. The present study, therefore, is based primarily on the first 222 samples mentioned above.

DISTRIBUTION AND ECOLOGY

As presently understood, the range of *G. minus* s. str. extends from eastern and central Pennsylvania southwestward to northwestern Georgia and northern Alabama and westward to central Tennessee, western Kentucky and south-central Indiana. This species is almost exclusively restricted to areas underlain by carbonate rocks. *G. minus* may also range from southwestern Illinois across southern Missouri and northern Arkansas to the extreme northeastern corner of Oklahoma (Hubricht and Mackin, 1940; Mackin, 1941; Hubricht, 1943; Hubricht, 1959; Minckley and Cole, 1963). However, further critical examination of material from this area, especially from the Ozark Plateau region, is needed before definite determinations can be made. The middle-western material tentatively referable to *G. minus* (*G. minus* s. lat.) is being studied currently by G. A. Cole (*in litt.*).

Throughout the eastern range of *G. minus*, the sympatric or syntopic occurrence of this species with any other species of *Gammarus* is extremely rare; the only notable exception is the sympatric association of *G. minus* and *G. bousfieldi* in Doe Run in Meade Co., Kentucky (Minckley and Cole, 1963). A similar situation of mutually exclusive ranges occurs in England, where overlapping ranges of species of freshwater *Gammarus* are uncommon (Hynes, 1955). This situation does not hold true, however, for the western Illinois-eastern Missouri area where there are overlapping ranges of four species of *Gammarus*: *G. troglophilus*, *G. acherondytes*, *G. pseudolimnaeus* and *G. minus* (s. lat.). Species pairs of *Gammarus* in this area may consist of *G. troglophilus*/*G. acherondytes* (restricted to Monroe and St. Clair Counties, Illinois), *G. minus* (s. lat.)/*G. pseudolimnaeus*, and occasionally *G. minus* (s. lat.)/*G. troglophilus*. The association of *G. minus* (s. lat.) with *G. pseudolimnaeus* in southern Illinois springs is rather common. Character displacement may influence the slight but subtle differences noted for populations of *G. minus* (s. lat.) from southwestern Illinois and farther west, which have so far complicated

the specific assignment of this material. The possibility that character displacement sometimes occurs when *G. minus* exists syntopically with other species of *Gammarus* should be investigated in greater detail. Minckley and Cole (1963) studied this possibility for *G. minus*/*G. bousfieldi* in Doe Run, Kentucky, but the evidence for character displacement in *G. minus* in this particular association was unclear and obscured by other factors (see Discussion and Conclusions section).

G. minus is generally limited in habitat to cave streams, springs and small spring-runs. Larger streams, ponds and even cave pools are seldom inhabited by this species. For example, Spring Creek in Greenbrier County, West Virginia is a large stream, principally derived from spring water. The springs that feed this creek contain large populations of *G. minus* but the creek itself does not. *G. minus* probably is excluded from larger streams by a number of ecological factors such as temperature differences, pO₂, available food, predation, cover and flow rate.

It is possible that spring forms of *G. minus* (Form III) disperse by washing downstream during late winter and spring flooding and subsequently migrate back upstream to the same or a different spring when the water level and flow rate return to normal. We observed a part of this postulated dispersal procedure during February 1967 in Pocahontas County, West Virginia. A large population of *G. minus* III occurs in a spring resurgence at the entrance to Overholts Blowing Cave. Observations throughout most of the year revealed that this population is limited to a narrow band extending downstream only a short distance from the cave entrance. However, during a late winter thaw in February 1967, we observed a number of individuals more than one-half mile downstream from the spring. Some of these individuals presumably migrate upstream later and re-populate the spring. Unfortunately, we have no quantitative data to indicate how many individuals are washed downstream during periods of high water or flooding or how many individuals subsequently migrate back upstream to a given spring. There are studies on upstream movement in amphipods which point out that not all species are able to migrate. In a paper on upstream movements of *Gammarus* in Doe Run, Kentucky, Minckley (1964) pointed out that *G. bousfieldi* could migrate *en masse* upstream, while the upstream movement of *G. minus* was much less obvious. Perhaps, on the contrary, there

is no appreciable upstream movement by *G. minus* to springs after flooding and the ability of this species to maintain itself in springs and headwaters depends on its high reproductive rate. The question has not yet been answered satisfactorily by either our own or Minckley's observations, and an investigation on this aspect of the animal's ecology might provide some interesting data. Nevertheless, Minckley (1963) did point out that very short upstream movements of *Gammarus* of one to two feet per day would assist in maintaining populations in headwaters and springs.

G. minus I is represented by populations in two well separated karst areas of the central Appalachian region of Virginia and West Virginia: the Great Savannah karst of south-central Greenbrier County, West Virginia in the Greenbrier Valley (New-Kanawha River drainage); and the Maiden Springs karst of southwestern Tazewell County, Virginia in the headwater region of the Clinch River (upper Tennessee River drainage). These two karst areas are separated by about 76 airline miles and several prominent mountains and ridges typical for this part of the Appalachians. Populations of *G. minus* (Forms II and III) occur in caves and springs located between and on the sides of these two areas.

G. minus I occurs in areas of extensive cave and karst development further characterized by integrated subsurface drainage. Therefore, we assume that limited dispersal by this species from one cave stream to another can take place through the underground conduits hypothesized to exist between caves in these areas. In the Great Savannah karst, which we have examined most carefully, *G. minus* I is the most common amphipod species in most cave streams. It is much more common than two other amphipods, *Stygonectes emarginatus* and *S. spinatus*, with which it is sometimes associated. Although common in cave streams, *G. minus* I is seldom encountered in rimstone pools located above streams or out of reach of potential flooding by streams. Over 100 rimstone pools were examined in Greenbrier Valley caves and only two of these contained specimens (a total of four) of *G. minus*. In marked contrast are the two troglobitic species of *Stygonectes* which were encountered in rimstone and other kinds of drip pools nearly 20 percent of the time and are apparently able to disperse interstitially by phreatic and vadose routes other than streams (Holsinger, 1967, 1969).

In comparison with *G. minus* I, *G. minus* II is found over a

much wider part of the central Appalachians and is known primarily from caves in Greenbrier, Monroe, Mercer, Randolph, Tucker and Pocahontas Counties in West Virginia; Giles and Tazewell Counties in Virginia; and Fayette County in Pennsylvania. With a few exceptions, this form is comparatively less abundant in cave streams than *G. minus* I, and usually occurs in caves representative of small to medium-sized subterranean drainage systems. On the other hand, this form is occasionally found in large cave systems such as Bone-Norman and Friars Hole in Greenbrier County and is rather common in caves of the well-developed karst of southern Pocahontas County (such as Swago Creek and Hillsboro areas).

Specimens of Form II also have been collected from caves on the periphery of the Great Savannah karst (viz., Grapevine and Fullers Caves) and of the Maiden Springs karst (viz., Lost Mill Caves). In the caves of Tucker, Randolph and Pocahontas Counties this form is more common (although often not very abundant) than any other amphipod species of this region. In some of these caves it is the only amphipod species recorded.

Of further ecological significance is the almost complete absence of *G. minus* from the cave streams of southwestern Virginia (especially in Lee, Scott and Russell Counties), eastern Tennessee, northwestern Georgia and northern Alabama. Some of the karst, caves and subterranean drainage complexes of this part of the Appalachians are developed to an extent comparable with those of Greenbrier and Tazewell Counties; yet, despite the common occurrence of *G. minus* in springs of this region and intensive collecting over a ten year period, this species is rarely found in these caves. The same situation is generally true of the caves of the adjacent Interior Low plateau region of southern Indiana, Kentucky and central Tennessee where some of the most extensive subterranean drainage systems in the world exist. A partial reason for the scarcity of *G. minus* in cave streams of these regions is probably its inability to compete successfully with troglobitic species of *Crangonyx*, *C. antennatus* and *C. packardii* (s. lat.). These two species of *Crangonyx* are distributed over an area extending from southwestern Virginia southwestward to Georgia, across northern Alabama, and north through central Tennessee, central Kentucky and into southern Indiana.

In the upper Tennessee River drainage basin, especially in the

Powell valley of southwestern Virginia, a concentrated investigation of caves has revealed that *C. antennatus* is a very common species that inhabits both drip pools and small streams (Holsinger, 1969). The cave-stream macrohabitat of this species appears to be similar to that of *G. minus* (I and II) of other areas. *C. packardii* (s. lat.) occupies essentially the same kind of habitat in some of the Interior Low plateau caves, especially those of central Kentucky and southern Indiana. The vagility of these two species of *Cran-gonyx*, combined with their ability to populate, often in large numbers, both pools and small streams, is probably indicative of their adaptive success and may be the major reason for the near exclusion of *G. minus* from caves of the same areas.

ANALYSIS OF VARIATION

A few minor structural variations were noted that apply to *G. minus* in general but not to any one morphological form. We will comment on these first. Minckley and Cole (1963) studied morphological variation in several populations of *G. minus* from northern Kentucky and observed differences in the amount of setation of the first four coxal plates and peduncular segments of the first antenna, occasional absence of calceoli on the second antenna of the male and minor size differences in sexually mature individuals. In general our studies revealed similar variation. In particular, we found that calceoli were occasionally absent on the second antenna of the male but were usually present in larger males. Like Minckley and Cole (1963), we examined the setal formula given by Bousfield (1958) i.e., peduncular segments 1, 2 and 3 with 1, 3-5 and 1 ventral groups of setae respectively, and found occasional but slight variation.

Finally, our observations revealed that the number of accessory flagellar segments of the first antenna may range up to 5 or 6 in some of the larger males. A slight range variation in number of accessory flagellar segments (from 3 to 6) also was recorded by Minckley and Cole (1963) but the differences between populations were not significant. In his diagnosis of *G. minus*, Bousfield (1958) gave a range of only 3 to 4 accessory flagellar segments, but his observations apparently were based on a small number of specimens.

We will next examine in detail those characters found by some earlier workers to differ among the three forms of *G. minus*. Shoemaker (1940) listed six characters diagnostic of his variety *tenuipes*: a) slender gnathopods and pereopods, especially the second joint of the last three pereopods; b) lateral lobes of the head with rounded corners; c) reduced number of spines on the urosome in some specimens; d) eyes greatly reduced and occasionally almost absent; e) inner ramus of the third uropod proportionately longer; and f) weaker and more delicate appearance of the whole animal. Hubricht (1943) stated the Forms I and II had longer antennae and a bluish color, whereas Form III had shorter antennae and a brownish color.

In order to properly analyze the validity of the "diagnostic" characters given above, we assigned all populations studied in detail to one of the three groups based on habitat. These included: 1) the large, well-integrated cave systems in Greenbrier and Tazewell Counties; 2) other caves, excluding those listed in (1); and 3) springs, including resurgences at cave entrances. Thus, in general, Habitats 1, 2 and 3 should correspond to *G. minus* I, II and III populations, respectively.

As will be seen below, a division into habitat groups is essential because any mixed population can be separated into groups based on morphology alone. This, however, does not reflect any biological phenomenon except that populations differ.

The amount of degeneration of the compound eye is undoubtedly the most striking difference among populations from the three habitat groups. The various stages of eye degeneration are shown in Figure 1; the extremes between individuals from Habitats 1 and 3 are shown in Figures 2 and 3. In most specimens from Habitat 1 the amount of eye degeneration has reached the extreme shown in Figure 3, and there are no discernible eye facets remaining. Occasionally, however, a specimen was found in Habitat 1 with a few facets completely formed. Moreover, a few specimens were found in Habitat 2 populations with as much eye degeneration as those from Habitat 1 populations.

The populations from Habitats 2 and 3 can be separated on the basis of the number of well-formed facets in the compound eye. By using specimens from various localities the following data were obtained:

Springs (Habitat 3): $N = 87$, median = 28, range = 14 to 42

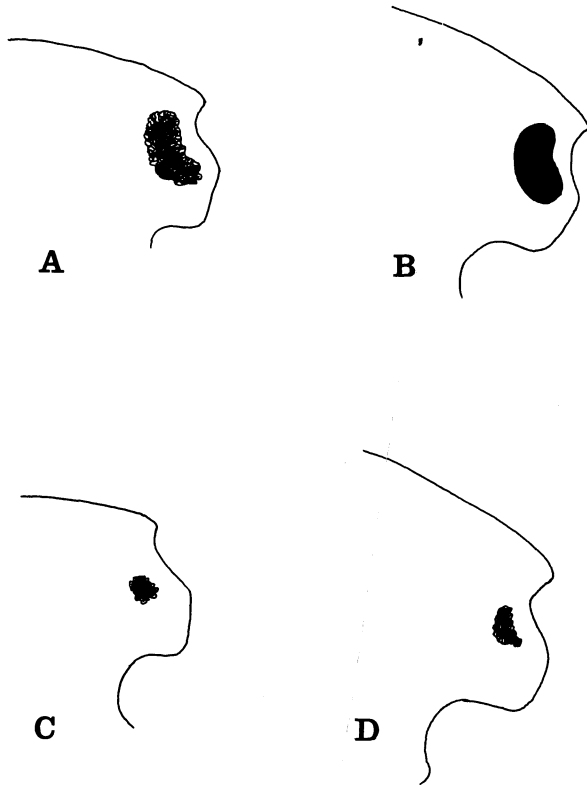


FIG. 1. Structure of the compound eye in mature males (11.00 mm) from four different populations of *Gammarus minus*. A) Tawneys Cave, Giles County, Virginia (Form II); B) spring in Washington County, Maryland (Form III); C) the Hole Cave, Greenbrier County, West Virginia (Form I); D) Bowens Cave, Tazewell County, Virginia (Form I).



FIG. 2. Head region of *Gammarus minus* (8.00 mm male) from Fort Spring, Greenbrier County, West Virginia. Upper, enlargement of compound eye showing individual facets.

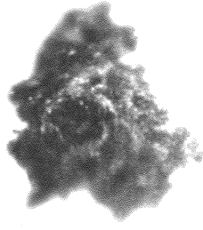


FIG. 3. Anterior region of *Gammarus minus* (12.00 mm male) from Benedicts Cave, Greenbrier County, West Virginia. Upper, enlargement of compound eye showing loss of individual facets.

Caves (Habitat 2): $N = 62$, median = 15, range = 0 to 33. The medians are significantly different ($P > 0.99$) using the distribution-free Mann-Whitney U test.

Many cases of asymmetry were noted in the amount of eye degeneration in individuals from all three habitat groups. One particularly striking example was a specimen from Coffmans Cave in Greenbrier County which had approximately 20 countable facets in one eye and none in the other. Despite slight variation and asymmetry noted above, differences in the amount of compound eye degeneration were more clear-cut than any other character investigated. A careful examination of numerous specimens did not support Shoemaker's contentions that the interantennal lobes of the head are more rounded in Form I animals or that the number of dorsal spines on the urosome is reduced.

Hubricht (1943) pointed out that spring populations are brownish in color and that cave populations are bluish. Although this observation is generally true, there is sometimes a greater variation in color than implied; spring forms tend to be brownish to brownish-green and occasionally brownish-red, while cave forms may vary from bluish to dull gray, and rarely, to almost colorless. For instance, the population from Linwood Cave (Habitat 2) Pocahontas County is especially light in color with some individuals almost colorless, but morphologically this population is intermediate between Forms II and III. Explanations for the reduction and/or loss of integumentary pigment in cave crustaceans are still incomplete, although a number of experimental studies have been published on this subject (Baldwin and Beatty, 1942; Beatty, 1942, 1949; Anders, 1956; Maguire, 1961). The integumentary pigment of amphipods is made up of various carotenoid-protein complexes; these animals are unable to synthesize the carotenoids and, therefore, must obtain them from the environment (Beatty, 1949; Maguire, 1961 and papers cited therein). Apparently pigmentation in many of the troglobitic forms is genetically controlled; hence exposure of these animals to environmental carotenoids does not cause integumentary pigment to reappear (Vandel, 1964). Troglonenes and troglonophiles vary in their coloration from surface to cave, so availability of usable carotenoid-containing foods may affect the development of carotenoid-based pigments in their integument (Maguire, 1961). Thus spring forms of *G. minus* with

apparently easy access to usable carotenoids in the form of abundant vegetable matter are brownish to brownish-green, while cave populations with apparently limited access to usable carotenoids undergo a reduction in pigmentation and are bluish to gray. If a difference in the availability of dietary carotenoids is the major factor in the development of pigment in *G. minus*, then any concomitant genetic influence on pigment production would be masked and very difficult to discern.

Both Shoemaker (1940) and Hubricht (1943) pointed out that *G. minus* I had proportionately longer and more slender appendages and hence a more fragile appearance than Form III, but it is probably the proportionately longer pereopods and antennae that give *G. minus* I the overall appearance of having a more delicate body and more slender appendages. These subjective observations are more apparent than real. Shoemaker (1940) also stated that the second joints (or bases) of the last three pereopods were more narrow than in the surface forms, but this is rare. The widths of the pereopod bases differ only slightly among representatives of the habitat groups as seen in Figure 4.

Some real differences among the three morphological groups in the proportionate lengths of certain appendages compared to body lengths suggests a trend toward allometry in the cave forms. To investigate the significance of these differences we made numerous measurements and treated the resulting data statistically.

Tables 1 and 2 give data for ratios of pereopod 7, uropod 3 and antenna 1 to total length for mature males from a variety of spring and cave populations. The ratios among the three habitat groups were statistically significant (Mann-Whitney U test) with two exceptions: a) uropod 3/total length is not significantly different in springs and caves (Habitat 2); b) pereopod 7/total length is only marginally significant ($P < 0.10$) in springs and caves (Habitat 2).

A general regression equation for appendage growth is $Y = aX^b + c$, where: Y is the dependent variable, i.e., antenna 1, pereopod 7 and uropod 3; b is the coefficient of allometry which equals 1 when no allometry occurs; X is the independent variable, i.e., total length; a is the slope of the regression line; c is the intercept on the ordinate. From this regression there are four ways in which the different ratios of dependent variable to independent variable might arise:

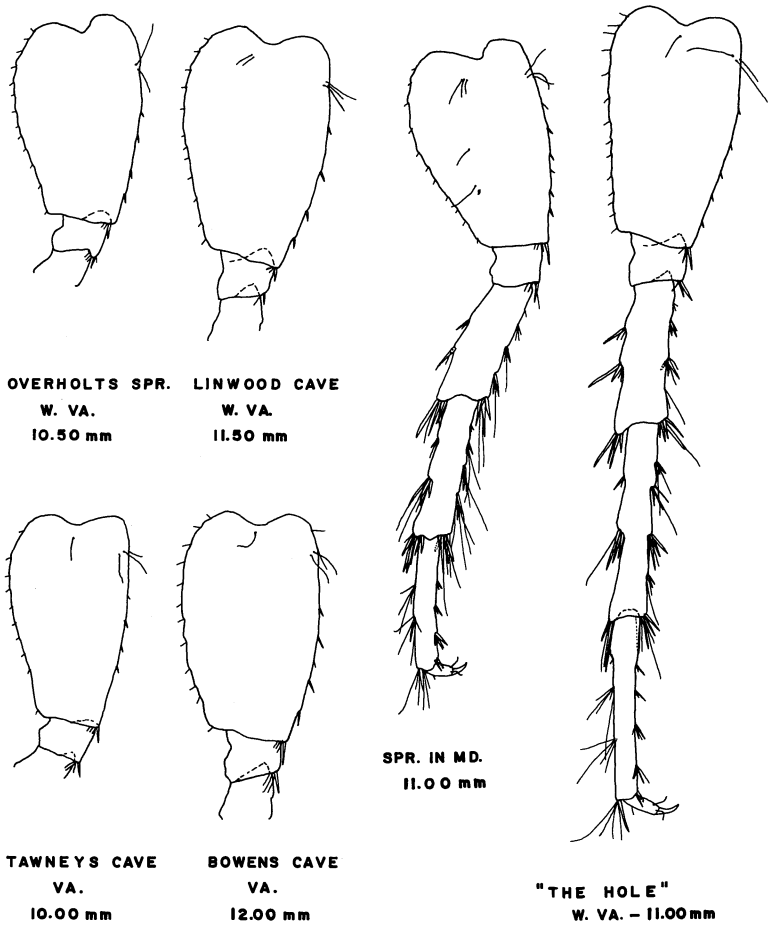


FIG. 4. Variation in structure of pereopod 7 in mature males from the indicated localities. Note difference in length but general similarity in structure of the pereopods of the males from the spring in Maryland (Form III) and the cave in West Virginia (Form I). Note also the slight variation in the shape and armature of the bases of the pereopods of males from different populations.

TABLE 1. Variation in the ratio of length of three different appendages (antenna 1, pereopod 7 and uropod 3) to length of body in mature males of 12 populations of *Gammarus minus*.

Cave Localities	Sample size	Mean length (mm)	Mean ratios		
			Antenna 1/body	Pereopod 7/body	Uropod 3/body
Higginbothams, Greenbrier Co., W.Va.	5	11.9	0.698	0.485	0.217
Buckeye Creek, Greenbrier Co., W.Va.	5	10.9	0.652	0.461	0.192
Grapevine, Greenbrier Co., W.Va.	5	11.8	0.655	0.489	0.187
Linwood, Pocahontas Co., W.Va.	5	12.3	0.590	0.426	0.175
Cave Hollow, Tucker Co., W.Va.	5	10.8	0.625	0.452	0.180
Benedicts, Greenbrier Co., W.Va.*	5	10.8	0.750	0.513	0.223
Ludington, Greenbrier Co., W.Va.*	5	10.4	0.674	0.485	0.188
McClungs, Greenbrier Co., W.Va.*	8	11.3	0.720	0.523	0.207
The Hole, Greenbrier Co., W.Va.*	12	12.3	0.707	0.494	0.200
Greenbrier, Greenbrier Co., W.Va.*	20	12.0	0.647	0.482	0.209
Hugh Young, Tazewell Co., Va.*	7	11.0	0.749	0.485	0.226
Bowens, Tazewell Co., Va.*	11	11.9	0.793	0.493	0.226

*Indicates Habitat 1 cave; the remaining populations are from Habitat 2 caves.

TABLE 2. Variation in the ratio of length of three different appendages to body length in mature males of 11 spring populations of *Gammarus minus*.

Spring Localities	Sample size	Mean length (mm)	Mean of ratios		
			Antenna 1/body	Pereopod 7/body	Uropod 3/body
Washington Co., Maryland	20	11.8	0.529	0.426	0.183
Smulton Spr., Centre Co., Pa.	10	10.1	0.576	0.449	0.188
Lancaster Co., Pa.	10	11.6	0.515	0.432	0.185
Tazewell Co., Va.	10	10.3	0.572	0.443	0.189
Maiden Spr., Tazewell Co., Va.	5	9.1	0.556	0.416	0.175
Spr. at Van, Lee Co., Va.	6	8.7	0.602	0.486	0.195
Windfields Spr., Bath Co., Va.	18	11.8	0.569	0.432	0.185
Sweet Sprs., Monroe Co., W.Va.	10	8.6	0.579	0.424	0.178
Overholt Spr., Pocahontas Co., W.Va.	11	9.6	0.613	0.452	0.190
Cold Spr., Monroe Co., W.Va.	10	9.7	0.586	0.443	0.191
Spr. on Mill Run, Tucker Co., W.Va.	10	11.2	0.570	0.459	0.166

1) Different ratios may reflect different coefficients of allometry. In particular, when b is less than 1 the dependent variable is increasing with a decreasing rate, and when b is greater than 1, the dependent variable is increasing with an increasing rate.

2) a , b and c may be the same for all groups, but when $c \neq 0$, changing the size of the independent variable, i.e., total length, will change the ratio.

3) Different ratios may reflect different slopes of the linear ($b = 1$) regression line.

4) Different ratios may reflect different intercepts.

In practice, solving for b in the above equation involves taking the logs of both sides which causes c to disappear. In order to find c by the usual methods, b must be considered to be equal to 1. Moreover, we will only consider a when b is assumed to be equal to 1. First, we will consider those dependent variables that show allometric growth. Those that do not show any allometry will be considered in terms of the last three possibilities listed above by using the equation $Y = aX + c$.

TABLE 3. The results of a test for allometry between the three habitat groups of *Gammarus minus*.

Independent variable	Dependent variable	Habitat†	b^*
total length	pereopod 7	3 (springs)	0.96 ± 0.08
"	"	2 (caves)	0.86 ± 0.33
"	"	1 (large caves)	0.98 ± 0.16
"	uropod 3	3 (springs)	0.87 ± 0.11
"	"	2 (caves)	0.81 ± 0.62
"	"	1 (large caves)	1.10 ± 0.22
"	antenna 1	3 (springs)	0.79 ± 0.12
"	"	Fort Spring (3)	1.01 ± 0.12
"	"	2 (caves)	0.81 ± 0.31
"	"	Coffmans Cave (2)	0.92 ± 0.12
"	"	1 (large caves)	0.83 ± 0.14
"	"	Benedict Cave (1)	0.83 ± 0.14
outer ramus of third uropod	inner ramus of third uropod	Fort Spring (3)	1.27 ± 0.09
		Coffmans Cave (2)	1.15 ± 0.10
		Benedict Cave (1)	1.15 ± 0.09

†Those habitats labeled 1, 2 and 3 were calculated using only mature males. For Fort Spring, Coffmans Cave and Benedicts Cave, all sizes and both sexes were used.

*If b is not significantly different from 1.0 there is no evidence for allometry.

The results of the analysis of allometry are given in Table 3. The only clear case of positive allometry where b is greater than 1 occurs in the regression of the inner ramus of the third uropod against the outer ramus of the third uropod, but not in the regression of body length and uropod 3. However, positive allometry occurs in all three habitat groups, not just in the Habitat 1 populations. There is a tendency for antenna 1 to display slightly negative allometry in all three habitat groups. Therefore, we can conclude that there are no differences in allometry which could explain differences in the appendage to body length ratios.

If all the populations are on the same regression line, then the total lengths of the cave populations must be greater than the spring populations, and the intercept c must be less than one or vice versa. Random samples from three populations in Greenbrier County were measured for total length. These populations were from Benedicts Cave (Habitat 1), Coffmans Cave (Habitat 2) and Fort Spring (Habitat 3). A Mann-Whitney U test was used to test for significant differences. The Benedicts Cave population was significantly larger ($P > 0.99$) than Coffmans Cave population; the latter, in turn, was significantly larger than the Fort Spring population ($P > 0.99$). This relationship also holds true if only mature males are considered. If the mean lengths of mature males from springs (Table 2) are compared with mean lengths of mature males from caves of either Habitat 1 or 2, the differences are significant ($P > 0.95$ by the Mann-Whitney U test), but differences between the two cave habitat groups are not significant. These results indicate that as we go from spring forms to cave forms the total length (independent variable) increases, thus effectively moving up the regression line. However, when we consider the actual intercepts of the regression lines (see Table 4) very few of them have negative intercepts. Only in the comparison of the inner and outer rami of the third uropod are all of the intercepts of the three groups negative, and this particular ratio is complicated by allometry.

There is no significant pattern toward a higher slope or higher intercept in cave populations as shown in Table 4. However, the slopes of the Habitat 1 populations tend to be higher for pereopod 7, uropod 3 and antenna 1. A pattern in the intercepts is much less clear.

This pattern may not hold true for spring populations in general. The problem can be seen by comparing the antenna 1 regression of

TABLE 4. Analysis of the changes in intercept and slope of linear regression where $Y = aX + c$.

Independent variable	Dependent variable	Habitat*	a	c
total length	pereopod 7	3 (springs)	0.43 ± 0.04	1.73
"	"	2 (caves)	0.39 ± 0.16	8.33
"	"	1 (large caves)	0.48 ± 0.08	2.15
"	uropod 3	3 (springs)	0.16 ± 0.02	2.39
"	"	2 (caves)	0.12 ± 0.11	3.02
"	"	1 (large caves)	0.22 ± 0.05	-1.34
"	antenna 1	3 (springs)	0.44 ± 0.06	13.10
"	"	Fort Spring (3)	0.58 ± 0.06	-2.30
"	"	2 (caves)	0.35 ± 0.22	33.82
"	"	Coffmans Cave (2)	0.56 ± 0.07	5.50
"	"	3 (large caves)	0.59 ± 0.18	11.90
"	"	Benedict Cave (1)	0.45 ± 0.08	7.60
outer ramus of third uropod	inner ramus of third uropod	Fort Spring (3)	0.73 ± 0.04	-4.20
		Coffman Cave (2)	0.67 ± 0.05	-2.90
		Benedict Cave (1)	0.63 ± 0.04	-2.50

*Those habitats labeled 1, 2 and 3 were calculated using only mature males. For Fort Spring, Coffmans Cave and Benedicts Cave, all sizes and both sexes were used.

all spring populations with the Fort Spring population, Habitat 2 populations with the Coffmans Cave population and Habitat 1 populations with the Benedicts Cave population. Although expected, there is little correspondence between these pairs. This is due in part to the fact that only mature males were used for the first member of each pair, therefore resulting in the large standard errors seen in Table 4.

It is also possible to spot check for differences in slope in the regression by comparing the ratios of various appendages to total length from two populations with approximately the same range in total lengths. This minimizes the disturbing influences of allometry and the differences due to different intercepts. The results of these comparisons are shown in Table 5, and, as indicated by these data, one almost always finds a significant difference whenever populations from mixed habitats are compared. Whenever two like populations are compared, there are significant differences between these pairs about one-third of the time.

TABLE 5. Mann-Whitney U test on the ratios of lengths of antenna 1, pereopod 7 and uropod 3 to total length for a variety of population pairs of *Gammarus minus*.

Population pair and habitat group	Antenna 1	Uropod 3	Pereopod 7
Spr., Washington Co., Md. (3)			
*The Hole Cave, W.Va. (1)	<.001	<.001	<.001
*McClungs Cave, W.Va. (1)			
Spr., Tucker Co., W.Va. (3)	<.001	<.001	<.001
*The Hole Cave, W.Va. (1)			
Spr., Bath Co., Va. (3)	<.001	<.001	<.010
*Bowens Cave, Va. (1)			
Spr., Bath Co., Va. (3)	<.001	<.001	<.0001
*Higginbothams Cave, W.Va. (2)			
Spr., Bath Co., Va. (3)	<.001	<.001	<.001
*Grapevine Cave, W.Va. (2)			
Spr., Bath Co., Va. (3)	<.001	<.005	N.S.
*Grapevine Cave, W.Va. (2)			
Spr., Washington Co., Md. (3)	<.001	<.025	N.S.
*Spr., Bath Co., Va. (3)			
Spr., Washington Co., Md. (3)	<.001	N.S.	N.S.
*The Hole Cave, W.Va. (1)			
Greenbrier Caverns, W.Va. (1)	<.005	N.S.	<.050
*Spr., Lee Co., Va. (3)			
Spr., Monroe Co., W.Va. (3)	N.S.	<.001	N.S.
Grapevine Cave, W.Va. (2)			
Higginbothams Cave, W.Va. (2)	N.S.	N.S.	N.S.
Cave Hollow Cave, W.Va. (2)			
Buckeye Creek Cave, W.Va. (2)	N.S.	N.S.	N.S.
*Ludington Cave, W.Va. (1)			
Benedicts Cave, W.Va. (1)	<.050	N.S.	<.005

*Indicates the population with higher ratios.

DISCUSSION AND CONCLUSIONS

On the basis of the observations and analyses given above, we have concluded that *G. minus* is a single, highly variable species. The careful examination of a number of diagnostic characters did not reveal a single character that would unequivocally divide this species into separate taxa. The former recognition of a separate and distinct variety *tenuipes* probably resulted from the failure of earlier workers to examine sufficient material from a wide variety of habitats.

As already pointed out, Minckley and Cole (1963) found measurable variations in populations of *G. minus* from northern Kentucky. When associated with *G. bousfieldi* in Doe Run, *G. minus* differed from the "typical" spring populations and the possibility of char-

acter displacement was implied. Morphological variation noted for Doe Run populations associated with beds of *Fissidens* (an aquatic moss) resulted from their association with the moss, since the same variation was noted in *G. minus* whether *G. bousfieldi* was present or absent (Minckley and Cole, 1963). Similarly, some of the variation we observed within populations from the various habitat groups (see Tables 1, 2 and 5) might also have been affected by variation in the nature of the habitat. The springs observed varied: some are fast-flowing like the Overholts Blowing Cave resurgence; some are small, seepage types while others are large, pond-like springs with considerable vegetation (such as Fort Spring). According to Houston (1960), differences in current velocity affect the size at maturity of *Gammarus pulex*. Similarly, current velocity may also affect the size at maturity of *G. minus* in both caves and springs, since flow rate in both kinds of habitats is often variable. Other environmental parameters of cave streams such as temperature, quantity of washed-in food and chemistry, are also variable and are often influenced by seasonal changes on the surface.

One question still largely unresolved is why populations of *G. minus* I are restricted to certain caves in two very specific areas. Are certain caves able to select out these extreme types while others are not? We cannot satisfactorily answer this question, especially in view of the fact that *G. minus* II occasionally occurs in large cave systems (such as Bone Norman Cave) and some of the *G. minus* II populations occupy caves immediately adjacent to or even hydrologically related to caves with Form I populations. It should be noted, however, that *G. minus* I does not inhabit smaller, semi-isolated caves and that the two areas inhabited by *G. minus* I contain large, well-integrated drainage systems. Bowens and Hugh Young Caves, although small in terms of human traverse, are a part of a large, underground drainage complex believed to resurge through Maiden Springs in Tazewell County. The most accessible part of this complex is Fallen Rock Cave which contains five or more miles of traversable passages. In terms of traversable size, however, some of the Great Savannah caves in Greenbrier County are considerably larger. Greenbrier Caverns, for example, has at least 18 miles of explorable passages. It would appear, then, that a large subterranean drainage system is a necessary prerequisite for the development of Form I morphology. Undoubtedly, selection is playing a role here, but exactly how it operates is not clear.

The variation in *G. minus* may be genetic or ecophenotypic, or influenced by both effects. It is apparent that the *G. minus* I morphology is composed of the extremes present in both *G. minus* II and III populations that are being selected for by certain, as yet undetermined, factors of the subterranean environment. Depending on circumstances, the same morphological change may be ecophenotypic or genetic; processes similar to those shown by Waddington's (1956) classical experiment on genetic assimilation of environmentally induced change in *Drosophila melanogaster* may explain some variation in *G. minus*.

If the three forms of *G. minus* are components of a single, variable species, one must accept the potential for gene exchange between population extremes. The opportunities for gene exchange are probably as great, or even greater, between certain spring and cave populations than they are between widely distributed spring populations. Almost any spring population is semi-isolated and such populations are best regarded as geographic isolates in the sense of Mayr (1963). If we consider physical barriers and drainage patterns in karst areas, migration from a spring into a cave or vice versa is easier to envision than migration from one spring to another. The latter event usually would be limited to the rather circuitous routes of surface streams, while the former could take place more directly. One means by which dispersal might occur between springs has already been suggested, but at best this method is limited and difficult to conceive of as a common event. Environmental conditions, rather than isolation, might play a more significant role in determining the form in a given habitat.

One of the problems encountered with *G. minus* in the central Appalachians was somewhat similar to that which Christiansen and Culver (1968) found in the cave collembolan *Pseudosinella hirsuta*. With this species, there was a striking parallelism and convergence in morphology in geographically isolated, highly cave-adapted populations. We found a similar situation with *G. minus* in two well-separated and isolated karst areas with similar habitats. The *tenuipes* form may be a convergent ecotype (see Dobzhansky, 1951) which occurs only under special environmental conditions and in the presence of proper genetic variants.

The present study is preliminary and it is obvious that there are a number of uninvestigated aspects pertinent to the overall problem which would provide interesting topics for future studies.

ACKNOWLEDGMENTS

A part of this study was supported by grants to Holsinger from the National Speleological Society Research Advisory Committee and the Old Dominion University Educational Foundation and by a grant to Culver from the Cave Research Foundation. We are grateful to the West Virginia Association for Cave Studies, Inc. (WVACS) for providing field assistance in Greenbrier County, and to Dr. Thomas E. Bowman for providing specimens from the Smithsonian Institution (United States National Museum). We thank the following persons for their assistance in the field and/or for their contribution of specimens: Roger Baroody, William Biggers, John and Martha Cooper, Richard Franz, Ronald Larue, Charles Maus, David Newson, John Rutherford and Paul Starr. We are also indebted to a number of landowners who kindly allowed us to visit caves on their property.

LITERATURE CITED

- Anders, Fritz. 1956. Über Ausbildung und Vererbung der Körperfarbe bei *Gammarus pulex* ssp. *subterraneus* (Schneider), einer normalerweise pigmentlosen Höhlenform des gemeinen Bachflohkrebses. *Zeit. indukt. Abstammungs-u. Vererbungslehre*, **87**: 567-579.
- Baldwin, E. and R. A. Beatty. 1942. The pigmentation of cavernicolous animals I. The pigments of some isopod Crustacea. *J. Exp. Biol.*, **18**: 136-143.
- Beatty, R. A. 1942. The pigmentation of cavernicolous animals II. Carotenoid pigments in the cave environment. *J. Exp. Biol.*, **18**: 144-152.
- 1949. The pigmentation of cavernicolous animals III. The carotenoid pigments of some amphipod Crustacea. *J. Exp. Biol.*, **26**: 125-130.
- Bousfield, E. L. 1958. Fresh-water amphipod crustaceans of glaciated North America. *Can. Field-Natur.*, **72**(2): 55-113.
- Christiansen, K. A. and D. C. Culver. 1968. Geographic variation and evolution in *Pseudosinella hirsuta*. *Evolution*, **22**: 237-255.
- Dobzhansky, Theodosius. 1951. *Genetics and the origin of species*. Columbia Univ. Press, New York, 364 p.
- Holsinger, J. R. 1967. Systematics, speciation, and distribution of the subterranean amphipod genus *Stygonectes* (Gammaridae). *U.S. Nat. Mus. Bull.*, **259**, 176 p.
- 1969. Biogeography of the freshwater amphipod crustaceans (Gammaridae) of the central and southern Appalachians pp. 19-50. *In* P. C. Holt [ed.] *The distributional history of the biota of the southern Appalachians. Part I: Invertebrates*. Virginia Polytechnic Inst. Press, Blacksburg, Va.
- Houston, K. 1960. Size of *Gammarus pulex* in fast- and slow-running streams. *Report Dauntsey's School Nat. Hist. Soc.*, 1959-1961, pp. 11-13.

- Hubricht, L. 1943. Studies on the Nearctic freshwater Amphipoda. III: Notes on the freshwater Amphipoda of eastern United States with description of ten new species. *Amer. Midl. Nat.*, **29**: 683-712.
- 1959. Malacostraca (Amphipoda), pp. 876-878. *In* W. T. Edmondson [ed.] Ward and Whipple's freshwater biology. John Wiley and Sons, New York.
- Hubricht, L. and J. G. Mackin. 1940. Descriptions of nine new species of fresh-water amphipod crustaceans with notes and new localities for other species. *Amer. Midl. Nat.*, **23**: 187-218.
- Hynes, H. B. N. 1955. Distribution of some fresh-water Amphipoda in Britain. *Verh. int. Ver. Limnol.*, **12**: 620-628.
- Mackin, J. G. 1941. A key to the species of Amphipoda of Oklahoma. *Proc. Oklahoma Acad. Sci.*, **21**: 29-30.
- Maquire, B. 1961. Regressive evolution in cave animals and its mechanism. *Texas J. Sci.*, **13**: 363-370.
- Minckley, W. L. 1964. Upstream movements of *Gammarus* (Amphipoda) in Doe Run, Meade County, Kentucky. *Ecology*, **45**(1): 195-197.
- Minckley, W. L. and G. A. Cole. 1963. Ecological and morphological studies on gammarid amphipods (*Gammarus* spp.) in spring-fed streams of northern Kentucky. *Occasional Papers of the C. C. Adams Center for Ecol. Stud.*, **10**: 1-35.
- Mayr, E. 1963. *Animal species and evolution*. Belknap Press, Cambridge, Mass., 797 p.
- Say, T. 1818. An account of the Crustacea of the United States. *J. Acad. Natur. Sci. Philadelphia*, **1**: 374-401.
- Shoemaker, C. R. 1940. Notes on the amphipod *Gammarus minus* Say and description of a new variety, *Gammarus minus* var. *tenuipes*. *J. Washington Acad. Sci.*, **30**: 388-394.
- Stoll, N. R. (Chairman), R. Ph. Dollfus, J. Forest, N. D. Riley, C. W. Sabrosky, C. W. Wright, R. V. Melville (Sec'y.) 1964. International code of zoological nomenclature. International Trust for Zoological Nomenclature, London, 176 p.
- Vandel, A. 1964. *Biospéologie*. Gauthier-Villars, Paris, 619 p.
- Waddington, C. H. 1956. Genetic assimilation of the bithorax phenotype. *Evolution*, **10**: 1-13.

INFORMATION FOR AUTHORS

REVIEW The Publications Committee of the Peabody Museum of Natural History reviews and approves manuscripts for publication. Papers will be published in approximately the order in which they are accepted; delays may result if manuscript or illustrations are not in proper form. To facilitate review, the original and one carbon or xerox copy of the typescript and figures should be submitted. The author should keep a copy.

STYLE Authors of biological papers should follow the *Style Manual for Biological Journals*, Second Edition (Amer. Inst. Biol. Sci.). Authors of paleontological manuscripts may choose to follow the *Suggestions to Authors of the Reports of the U.S. Geological Survey*, Fifth Edition (U.S. Govt. Printing Office).

FORM Maximum size is 80 printed pages including illustrations (= about 100 manuscript pages including illustrations). Manuscripts must be typewritten, with wide margins, on one side of good quality 8½ x 11" paper. *Double space everything. Do not underline anything except genera and species.* The editors reserve the right to adjust style and form for conformity.

TITLE Should be precise and short. Title should include pertinent key words which will facilitate computerized listings. Names of new taxa are not to be given in the title.

ABSTRACT The paper must begin with an abstract. Authors must submit completed BioAbstract forms; these can be obtained from the *Postilla* editors in advance of submission of the manuscripts.

NOMENCLATURE Follow the International Codes of Zoological and Botanical Nomenclature.

ILLUSTRATIONS Must be planned for reduction to 4 x 6½" (to allow for running head and two-line caption). If illustration must go sideways on page, reduction should be to 3¾ x 6¾". All illustrations should be called "Figures" and numbered in arabic, with letters for parts within one page. It is the author's responsibility to see that illustrations are properly lettered and mounted. Captions should be typed double-spaced on a separate page.

FOOTNOTES Should not be used, with rare exceptions. If unavoidable, type double-spaced on a separate page.

TABLES Should be numbered in arabic. Each must be typed on a separate page. Horizontal rules should be drawn lightly in pencil; vertical rules must not be used. Tables are expensive to set and correct; cost may be lowered and errors prevented if author submits tables typed with electric typewriter for photographic reproduction.

REFERENCES The style manuals mentioned above must be followed for form and for abbreviations of periodicals. Double space.

AUTHOR'S COPIES Each author receives 50 free copies of his *Postilla*. Additional copies may be ordered at cost by author when he returns galley proof. All copies have covers.

PROOF Author receives galley proof and manuscript for checking printer's errors, but extensive revision cannot be made on the galley proof. Corrected galley proof and manuscript must be returned to editors within seven days.

COPYRIGHT Any issue of *Postilla* will be copyrighted by Peabody Museum of Natural History only if its author specifically requests it.