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A REVISION IN THE SPHAEROMID GENUS GNORIMOSPHAEROMA MENZIES (CRUSTACEA: ISOPODA) ON THE BASIS OF MORPHOLOGICAL, PHYSIOLOGICAL AND ECOLOGICAL STUDIES ON TWO OF ITS "SUBSPECIES"

J. A. RIEGEL

Department of Zoology, University of California, Davis, California

A REVISION IN THE SPHAEROMID GENUS GNORIMOSPHAEROMA MENZIES (CRUSTACEA: ISOPODA) ON THE BASIS OF MORPHOLOGICAL, PHYSIOLOGICAL AND ECOLOGICAL STUDIES ON TWO OF ITS "SUBSPECIES"

J. A. RIEGEL [1]

Department of Zoology, University of California, Davis, California

During a recent study into the physiology of osmoregulation in sphaeromid isopods (Riegel, 1959), an interesting problem was brought to light concerning the taxonomy of *Gnorimosphaeroma oregonensis* (Dana, 1852). Menzies (1954a) split the species into two subspecies, *lutea* and *oregonensis*. *G. o. oregonensis* was described as a typical intertidal bay form, inhabiting the undersides of rocks in waters whose salinity approached that of normal sea water. *G. o. lutea* was described as a creek or pond dweller confined to waters of low salinities and often associated with mud and vegetation. Differences in osmoregulatory ability and ecological requirements, as well as morphological differences, lead the writer to doubt the validity of Menzies' subspecies.

Riegel (1959) performed experiments on specimens from fresh-water, estuarine and bay populations of *Gnorimosphaeroma oregonensis* to test their osmoregulatory abilities. He found that specimens of the bay form [= *G. o. oregonensis* (Dana)] could neither regulate their body fluid concentration within viable limits nor survive for longer than a few days in fresh water. Specimens of the estuarine and fresh-water forms of *G. oregonensis* (= *G. o. lutea* Menzies 1954) were able to regulate their body fluid concentrations within viable limits and survive for over three weeks in salinities ranging from fresh water to 125 per cent sea water. Specimens of the fresh-water form, when in 50 per cent sea water or less, could maintain significantly higher body fluid concentrations than could specimens of the estuarine form. All forms of *G. oregonensis* were found to regulate hyper-osmotically in dilute media and hypo-osmotically in salinities just below or above normal sea water.

The present paper reports the results of experiments and observations designed to clarify the taxonomic position of the "subspecies" of *Gnorimosphaeroma oregonensis* described by Menzies (1954a). The differences which prompted the unofficial separation of that species into three habitat groups (*i.e.*, estuarine, fresh-water and bay forms) in a previous paper (Riegel, 1959) are no longer under primary consideration, so to avoid confusion, Menzies' subspecific names will be used throughout the balance of this paper.

Methods

Experiments were conducted to determine the effect of salinity on the morphology of *Gnorimosphaeroma oregonensis lutea* and *G. o. oregonensis*. One

[1] Present address: Department of Zoology, State College of Washington, Pullman, Washington.

hundred young of each "subspecies," newly emerged from the brood pouch, were placed in separate finger bowls containing rocks and normal sea water. The normal sea water in which the young *G. o. oregonensis* were placed was diluted over a period of two weeks to ten per cent sea water. Controls consisting of newly-emerged young of both "subspecies" were kept in normal habitat water, but otherwise under identical conditions. The young isopods were fed slices of frozen shrimp occasionally, and the water was changed weekly.

In another experiment, more than two hundred ovigerous females (plus over a hundred males and immature individuals) of *Gnorimosphaeroma oregonensis lutea* were placed in a large plastic tub containing normal sea water and otherwise simulating the natural habitat as closely as possible. The "brood pouches" of the females contained eggs and embryos in all stages of development. The animals were fed slices of frozen shrimp occasionally. Samples of twenty young were removed from the tub each week (for three months) and examined for possible morphological differences created by the high salinity. This experiment was done in order to subject specimens of *G. o. lutea* to high salinity as early in their embryological development as possible, in case the effect of salinity (as measured in the first experiment, described above) is no longer exerted on the morphology of the animals after they leave the brood pouch.

To ascertain to what extent (if any) populations of *Gnorimosphaeroma oregonensis lutea* and *G. o. oregonensis* intergrade ecologically, the writer made extensive surveys in San Francisco Bay, Tomales Bay, and coastal inlets from Half Moon Bay, San Mateo County, to Stillwater Cove (near Fort Ross), Sonoma County, California.

As the result of handling large numbers of *Gnorimosphaeroma oregonensis lutea* and *G. o. oregonensis,* the writer observed that the body in the latter form seemed relatively broader than in the former form. Therefore, measurements of length and width were taken of random samples of the two "subspecies" from various localities and salinities. From these measurements, length/width ratios were calculated and analyzed statistically.

Results

The newly-emerged young of *Gnorimosphaeroma oregonensis lutea* and *G. o. oregonensis* died slowly over a six-week period. That their deaths were not due to salinity alone is indicated by the fact that the controls also died slowly. However, during the six-week period, some growth was detected (both by increase in size and the presence of cast-off exoskeletons), but no changes in the morphology of the animals were seen.

The young removed from the plastic tub in which were kept the ovigerous female *Gnorimosphaeroma oregonensis lutea* all showed the typical morphology of that "subspecies." Of particular significance was the fact that young which underwent their entire development in normal sea water showed no change from the typical morphological configuration of *G. o. lutea.* These were young hatched from eggs which were in the "brood pouches" of the females when the females were originally placed in normal sea water. At the end of the experiment, no ovigerous females were found in the plastic tub and many of the young were half grown (3–5 mm. long).

The results of surveys designed to determine the degree of ecological intergradation between *Gnorimosphaeroma oregonensis lutea* and *G. o. oregonensis* were as follows: *G. o. oregonensis* was found in only three locations, Stillwater Cove, Point San Quentin in San Francisco Bay, and Tomales Bay State Park in Tomales Bay. *G. o. lutea* was found in Pilarcitos Creek draining into Half Moon Bay, several creeks draining into Tomales Bay, and in the Napa River and several creeks draining into the northern end of San Francisco Bay. In only one location did the two "subspecies" occur in the same general area, which was at Tomales Bay State Park. At the other locations cited above for *G. o. lutea,* conditions were probably not suitable for *G. o. oregonensis,* since the substrate was either muddy or sandy and lacked the rocks and loose rubble which characterize the habitats from which the writer has collected the latter form. At Tomales Bay State Park, where the two "subspecies" occurred together, there was a sharp break in their respective habitats. *G. o. lutea* was found in a small creek among the vegetation and under rocks. Little more than 50 feet away, but in the intertidal area of the rocky beach, *G. o. oregonensis* was collected from under rocks and among the loose rubble. The only known barrier present was the very dilute salinity of the creek water (fresh to taste), which would probably prevent *G. o. oregonensis* from entering the creek. Since *G. o. lutea* is capable of living in salinities approaching normal sea water, there appeared to be no salinity barrier to its colonization of the intertidal area. However, no specimens referable to *G. o. lutea* were found in the *G. o. oregonensis* habitat, and no specimens of *G. o. oregonensis* were found in the *G. o. lutea* habitat. At Stillwater Cove, *G. o. oregonensis* were collected in the loose rubble and under stones intertidally. There was a small stream running into the cove, which appeared suitable for habitation by *G. o. lutea,* but that form was not found there.

From measurements of the body length and width of random samples of ten specimens of each of the two "subspecies," the following ratios were found. The length/width ratio of the body of *Gnorimosphaeroma oregonensis oregonensis* averages 1.64 ± 0.021, with a range of 1.50 to 1.75. The length/width ratio of the body of *G. o. lutea* averages 1.84 ± 0.018, with a range of 1.70 to 1.96. The mean differences were found to be highly significant statistically ($t = 7.29$). Subsequent checks of specimens of both "subspecies" from different localities and salinities made since the above measurements were taken bear out the decided separation in the range of length/width ratios between *G. o. oregonensis* and *G. o. lutea.*

Discussion

Osmoregulatory ability in relation to ecology

In general, the osmoregulatory abilities of the various experimental groups reported in a previous paper (Riegel, 1959) and summarized in the present paper, agree well with their habitat and distribution according to salinity tolerance.

The fresh-water form of *Gnorimosphaeroma oregonensis lutea* is a good osmoregulator, which would be expected of a successful invader of fresh water. One barrier to complete adaptation to fresh water, namely, the ability to reproduce and rear young in that medium, possibly has been circumvented by this form. The brood pouches of most isopods are formed by flaps (oöstegites) projecting medially from the bases of four pairs of thoracic legs. The developing eggs and embryos

are held between the overlapping flaps and the sternum. However, in the Sphaeromidae, the eggs and embryos are carried in various types of internal "brood pouches" (Menzies, 1954b) which in *G. oregonensis* (*sensu lato*) are separated from the body fluid only by a thin and presumably permeable membrane. Thus, by regulating the body fluids osmotically, the sphaeromid probably can regulate the osmotic environment of the eggs and embryos. Young *G. o. lutea* which hatched out in the laboratory survived for several weeks in fresh water. Whether reproduction in fresh water is dependent upon the internal "brood pouch" of *G. o. lutea* or due to osmoregulatory ability or osmotic resistance of its embryos and eggs is not known.

Populations of *Gnorimosphaeroma oregonensis lutea* living in brackish water must adjust to salinity variation of at least two types: (1) Daily fluctuations in salinity of relatively short duration during the tidal cycles, and (2) seasonal fluctuations due to rainfall and runoff from melting snow. Measurements of habitat salinity and body fluid concentration changes during a portion of a tidal cycle (Riegel, 1959) showed the pattern of salinity fluctuations to which an estuarine population of *G. o. lutea* adapted. Between a period from low to high tide, the salinity of the habitat varied from fresh water (0.27% sea water) to 65 per cent sea water. During the same period, the body fluid concentrations of the resident *G. o. lutea* varied from 50 per cent sea water (in fresh water) to 70 per cent sea water (in 65% sea water). Another population of the "subspecies" which lived in a pond situation away from tidal salinity influences had to adjust to salinities as high as 60 per cent sea water during the late summer and fall, and as low as ten per cent sea water during the winter and spring periods of rain and heavy runoff from melting snow.

The internal "brood pouch" of *Gnorimosphaeroma oregonensis* living in brackish water is of possible value as a "buffering" mechanism to prevent damage to eggs and embryos by extreme fluctuations in the salinity of the habitat, especially in the lower salinity ranges.

Gnorimosphaeroma oregonensis oregonensis has never been collected from salinities approaching fresh water. The lowest salinity recorded in its habitat was about 12 per cent sea water, which occurred during a period of particularly heavy rains; presumably, this form is capable of surviving for short periods in such low salinities. Further, it is improbable that the animals must ever endure prolonged exposure to low salinity. During low tide, they are always found down in the coarse rubble on the beach. During conditions of low salinity in the general habitat, it is probable that in their microhabitat the salinities are higher due to water trapped by spaces in the rubble, leaching of residual salts from the rubble and evaporation.

Osmoregulation and other factors in relation to systematics

As mentioned previously, Menzies (1954a) split *Gnorimosphaeroma oregonensis* into two subspecies, *G. o. oregonensis* and *G. o. lutea.* However, because of differences in their osmoregulatory physiology and habitat preference, the validity of Menzies' subspecies is questioned by the writer.

The biological species is defined by Mayr *et al.* (1953, p. 313) as consisting of "groups of actually (or potentially) interbreeding natural populations which are reproductively isolated from other such groups." Mayr *et al.* define a subspecies (p. 314) as "a geographically defined aggregate of local populations which differs

taxonomically from other such subdivisions of the species." Let us analyze *Gnorimosphaeroma oregonensis* in the light of the above concepts.

Gnorimosphaeroma oregonensis occupies a very extensive range from Alaska to central California. From the distribution records (Menzies, 1954a), it appears that the species is broken up into fresh-water, estuarine and intertidal bay populations throughout its range. Barring the unlikely occurrence of multiple local evolutions, it is probable that the same mechanism(s) has created the *G. o. lutea* and *G. o. oregonensis* "subspecies" over the entire range. Therefore, at least three possibilities concerning the origin of the various forms present themselves. (1) The bay and estuarine-fresh-water forms separated long ago (in the sense of geological time) and both are still capable of distributing themselves across open ocean barriers. (2) The bay form has given rise to the estuarine-fresh-water form recently (or *vice versa*). (3) The morphological, physiological, and ecological differences between the two "subspecies" are salinity-induced and the two forms represent ecotypes of the same species. If the first alternative is true, the two "subspecies" are probably separate species. If the second alternative is true, they may be true subspecies.

There are only two apparent morphological differences between *Gnorimosphaeroma oregonensis oregonensis* and *G. o. lutea*—a difference in the morphology of the pleotelson (Fig. 1) and a difference in body proportions. According to Menzies (1954a), the third pleonite in *G. o. lutea* does not reach the lateral border of the pleotelson, but in *G. o. oregonensis* it does. Examination by the writer of several hundred specimens of each form taken from several localities and various salinities confirms Menzies' observation and shows also that the difference in pleotelson structure is remarkably consistent. Further, there were no intergrades with respect to this diagnostic character. The writer observed that the body in *G. o. oregonensis* seemed relatively broader than in *G. o. lutea.* Measurements of body length and width of specimens of the two "subspecies" from various localities and salinities bore out this observation and showed that there is a significant separation between the two forms in the average length/width ratio of the body.

The osmoregulatory abilities of the two supposed subspecies show striking differences. *Gnorimosphaeroma oregonensis oregonensis* was unable to survive in fresh water, at least under experimental conditions (see Riegel, 1959). *G. o. lutea* lived for several days in all salinities under the experimental conditions.

The ecological differences between the two "subspecies" are well-marked and possibly associated, at least in part, with the above-mentioned physiological difference. *Gnorimosphaeroma oregonensis oregonensis* lives intertidally in bays. During periods of low tide, it remains down in the coarse rubble on the beach, where it is out of water, but in very moist conditions. During high tide, it leaves its hiding places and presumably forages for food. *G. o. lutea* lives in estuaries and fresh-water streams and ponds. It is usually associated with mud and vegetation, but occasionally it may be collected from rocky bottoms of small streams. It lives among the roots of aquatic plants and in cracks in mud, rock, wood, and in burrows made by other animals. Of special interest in regard to the ecological separation of the two forms is the fact that *G. o. lutea* is fully capable of occupying the *G. o. oregonensis* habitat (taking into consideration only the former form's salinity

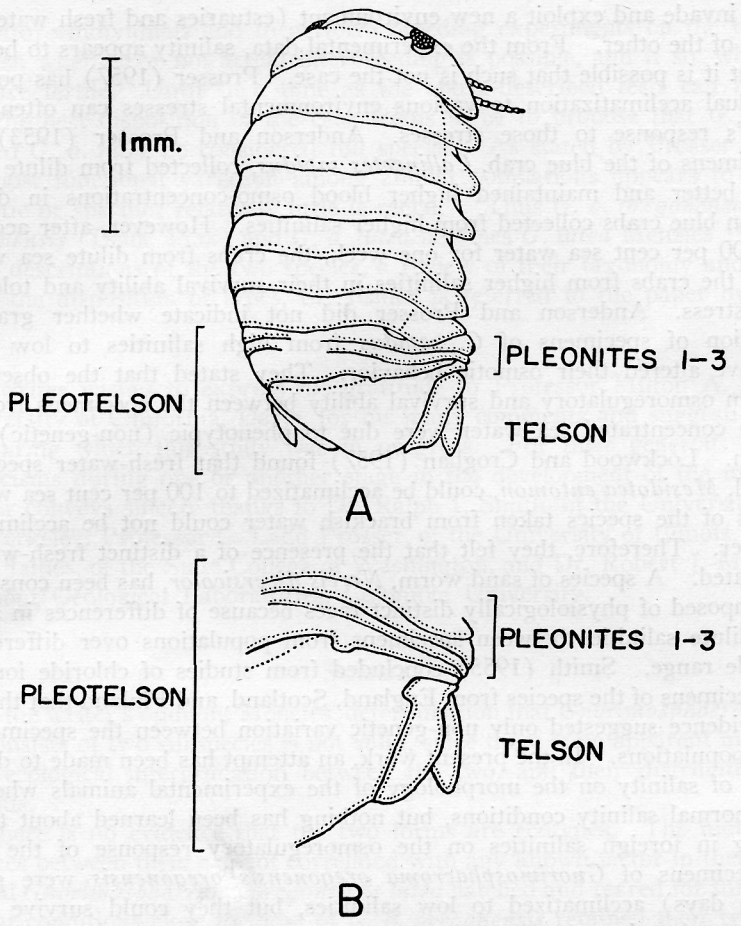

FIGURE 1. A. Diagram of *Gnorimosphaeroma oregonensis lutea* showing the whole animal and the details of its pleotelson morphology. B. Diagram of the pleotelson morphology of *Gnorimosphaeroma oregonensis oregonensis* (A and B after Menzies, 1954a).

tolerance), but it does not do so. Further, as far as can be ascertained, there are no natural hybrids between the two forms.

The osmoregulatory and ecological characteristics of *Gnorimosphaeroma oregonensis oregonensis* and *G. o. lutea* have been considered as adaptive in the sense of Allen (quoted by Pantin, 1932), who suggested that adaptation be measured by survival. From the teleological vantage point afforded by the experiments and observations presented in this paper, it appears that the ability of the two "subspecies" to survive within their respective habitats is correlated with their ability to osmoregulate and survive in various experimental dilutions and concentrations of sea water. Caution must be exercised in making statements concerning the actual barrier(s) or "selective factor(s)" which has permitted one form of *G. orego-*

nensis to invade and exploit a new environment (estuaries and fresh water) to the exclusion of the other. From the experimental data, salinity appears to be a major factor, but it is possible that such is not the case. Prosser (1957) has pointed out that gradual acclimatization to various environmental stresses can often alter an organism's response to those stresses. Anderson and Prosser (1953) showed that specimens of the blue crab, *Callinectes sapidus,* collected from dilute salinities survived better and maintained higher blood osmo-concentrations in dilute sea water than blue crabs collected from higher salinities. However, after acclimatization to 100 per cent sea water for one week, the crabs from dilute sea water approached the crabs from higher salinities in their survival ability and tolerance to osmotic stress. Anderson and Prosser did not indicate whether gradual acclimatization of specimens of *C. sapidus* from high salinities to low salinities would have altered their osmotic behavior. They stated that the observed differences in osmoregulatory and survival ability between the specimens from dilute and more concentrated sea water were due to phenotypic (non-genetic) osmotic adaptation. Lockwood and Croghan (1957) found that fresh-water specimens of the isopod, *Mesidotea entomon,* could be acclimatized to 100 per cent sea water, but specimens of the species taken from brackish water could not be acclimatized to fresh water. Therefore, they felt that the presence of a distinct fresh-water race was indicated. A species of sand worm, *Nereis diversicolor,* has been considered as being composed of physiologically distinct races because of differences in ability to tolerate dilute salinities between specimens from populations over different parts of its wide range. Smith (1955) concluded from studies of chloride ion regulation of specimens of the species from England, Scotland, and Finland that the experimental evidence suggested only non-genetic variation between the specimens from different populations. In the present work, an attempt has been made to determine the effect of salinity on the morphology of the experimental animals when raised under abnormal salinity conditions, but nothing has been learned about the effect of rearing in foreign salinities on the osmoregulatory response of the animals. Adult specimens of *Gnorimosphaeroma oregonensis oregonensis* were gradually (over ten days) acclimatized to low salinities, but they could survive only for less than two days in ten per cent sea water or less.

Referring to the three stated alternatives—are *Gnorimosphaeroma oregonensis oregonensis* and *G. o. lutea* subspecies, ecotypes, or separate species?—the writer presents the following conclusions. The lack of morphological and ecological intergrades between the two forms and their apparently discontinuous distribution rule out, in the writer's opinion, the possibility that they are true subspecies. Since there is no evidence, thus far encountered, to indicate that *G. o. oregonensis* and *G. o. lutea* are ecotypes of the same species, only a remote possibility persists that such is the case. The morphological differences between them are not correlated with any known factor in their environment, which is perhaps not to be expected, but some adaptive relationship is commonly seen in ecotypes. Further, specimens of *G. o. lutea* hatched and reared in habitat conditions close to those of *G. o. oregonensis* retained (at least for three months) their typical morphological configuration. Therefore, the writer is of the opinion that the two "subspecies" are actually species. To establish beyond all question their status as true species, it would be necessary to extend salinity tolerance tests on early developmental stages

and immature individuals and to perform breeding experiments on the two forms to establish whether or not actual interbreeding is possible, and if so, whether the hybrids are fertile. Until such time as the above-described tests can be accomplished, the best disposition of the case seems to be to propose that *G. o. oregonensis* and *G. o. lutea* be considered full species. As stated by Prosser (1957, p. 363), "most functional variation among animal populations appears to be either non-genetic or specific; relatively little is racial." Thus *G. o. oregonensis* becomes *G. oregonensis* (Dana, 1852) and *G. o. lutea* becomes *G. lutea* Menzies 1954. For complete descriptions of the two species, a review of their taxonomy and distribution and the disposition of types, the reader is referred to the paper by Menzies (1954a).

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Summary

1. In the writer's opinion, Menzies' determination that *Gnorimosphaeroma oregonensis* consists of two subspecies is not valid, since there is no apparent morphological and ecological intergradation between the two, and their distribution is discontinuous.

2. There is no evidence that the two forms are ecotypes. The morphological differences between them are not correlated with any known factor in their environment, and *Gnorimosphaeroma oregonensis lutea* hatched and reared for three months in habitat conditions close to those of *G. o. oregonensis* retained their typical morphological configuration.

3. It is the opinion of the writer that until such time as extensive rearing and breeding tests can be performed, it is best to propose the elevation of the two subspecies to full species status.

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[2] It seems only fair to state that Dr. Menzies is not in agreement with the conclusions drawn in this paper concerning the elevation of *Gnorimosphaeroma oregonensis oregonensis* and *G. o. lutea* to species status.

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