United States
Environmental Protection
Agency

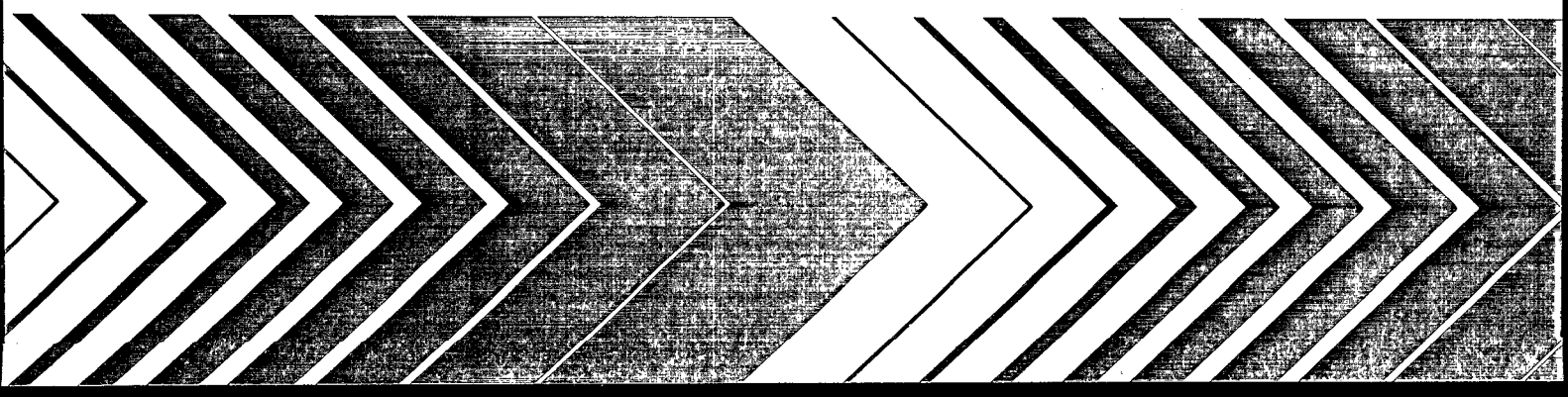
Environmental Monitoring and
Support Laboratory
Cincinnati OH 45268

EPA/600/4-86/032
September 1986

Research and Development

EPA

Taxonomy of *Ceriodaphnia* (Crustacea: Cladocera) in U.S. Environmental Protection Agency Cultures



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TAXONOMY OF CERIODAPHNIA (CRUSTACEA:CLADOCERA) IN U.S. ENVIRONMENTAL PROTECTION AGENCY CULTURES

by

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FOREWORD

Environmental measurements are required to determine the quality of ambient water, the character of effluents, and the effects of pollutants on aquatic life. The Environmental Monitoring and Support Laboratory - Cincinnati (EMSL-Cincinnati) conducts research to develop, evaluate, and promulgate methods to:

- Measure the presence and concentration of physical, chemical, and radiological pollutants in water, wastewater, bottom sediments, and solid waste.
- Concentrate, recover, and identify enteric viruses, bacteria, and other microorganisms in water.
- Measure the effects of pollution on freshwater, estuarine, and marine organisms, including the phytoplankton, zooplankton, periphyton, macrophyton, macroinvertebrates, and fish.
- Automate the measurement of physical, chemical, and biological quality of water.
- Conduct an Agency-wide quality assurance program to assure standardization and quality control of systems for monitoring water and wastewater.

Ceriodaphnia is a small relative of Daphnia that is currently being used to evaluate the chronic toxicity of pollutants to freshwater organisms. A chronic toxicity test employing this organism was included in the EMSL-Cincinnati manual, "Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms," EPA-600/4-85-014, which went to press in December, 1985.

During the initial development and field validation of the Ceriodaphnia chronic toxicity test, the organisms being cultured in the various Agency and private sector laboratories were tentatively identified as Ceriodaphnia reticulata, but as the use of the test became more widespread, there was increasing uncertainty and controversy about their true identity. Since the correct identification of test species is vital to the toxicity evaluation program, it was important to examine the issue promptly. To resolve this problem, Dr. Berner, the leading U.S. expert on Ceriodaphnia taxonomy, was engaged by EMSL-Cincinnati to examine organisms from Agency cultures. It was determined that the correct identification was Ceriodaphnia dubia.

This Report contains many excellent scanning electron micrographs and drawings of the specimens examined, and will prove very useful to biologists in confirming the identity of the organisms used in toxicity tests.

Robert L. Booth
Director
Environmental Monitoring and Support
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ABSTRACT

This study investigated the taxonomy of three groups of the cladoceran genus Ceriodaphnia in cultures being used by the U.S. Environmental Protection Agency. One taxonomic group, having heavy, triangular denticles in a pecten on the postabdominal claw and very short male antennules, was identified as C. reticulata (Jurine 1820). The second group, with a heavy, setulated pecten on the claw and long male antennules was identified as C. dubia Richard 1894. The third group was taxonomically nearly identical to C. dubia except that the claw pecten of females sometimes had ovate, sharp teeth rather than comb-like setules, depending upon culture conditions. This was determined to be a hitherto undescribed phenotypic variant of C. dubia, and is designated as C. dubia, toothed-pecten variety. Specimens of this form have been found in populations of C. dubia collected in the U. S. west of the Mississippi River.

Similarities in the general morphology, postabdomens, and ephippia of C. reticulata and C. dubia suggest that they are evolutionarily closely related and might be able to hybridize and produce offspring having an ovate-toothed pecten like that of the C. dubia variant. Experiments designed to test this possiblity were inconclusive although two successful interspecific matings were observed. It is suggested that the relationship between these two Ceriodaphnia could be further elucidated by study of more extensive field samples, and by interspecific breeding experiments that include hatching of hybrid young from ephippia and study of their taxonomy and fertility.

CONTENTS

FIGURES

ACKNOWLEDGMENTS

This work was supported, in part, by a contract from the U. S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Newtown Facility, Newtown, OH, and by a Faculty Research Grant-in-Aid from Temple University. It was mostly done while the author was a Visiting Scholar at Harvard University, where H. Levi kindly provided her with laboratory space in the Department of Invertebrates, Museum of Comparative Zoology.

The author is indebted to P. Lewis for suggesting this study, for patiently maintaining cultures, and for supplying materials for the experiments at the Newtown EPA Facility. W. B. Horning, II, of the same facility, kindly arranged for funding and gave encouragement. W. Peltier and K. Lamott were especially helpful in culturing and providing samples of the morph that turned up at the Athens, GA, EPA laboratory. D. Mount and T. Norberg are thanked for sending specimens from the EPA Duluth laboratory.

All those investigators who sent samples or let the author search through their collections in the course of this study are especially appreciated. Thanks go to the curators who loaned specimens from the Lilljeborg Collection, Uppsala; the British Museum (Natural History); and the U.S. Natural History Museum.

Lastly, E. Seling and K. Moskowicz are appreciated for their expertise in operation of the scanning electron microscope used in this study.

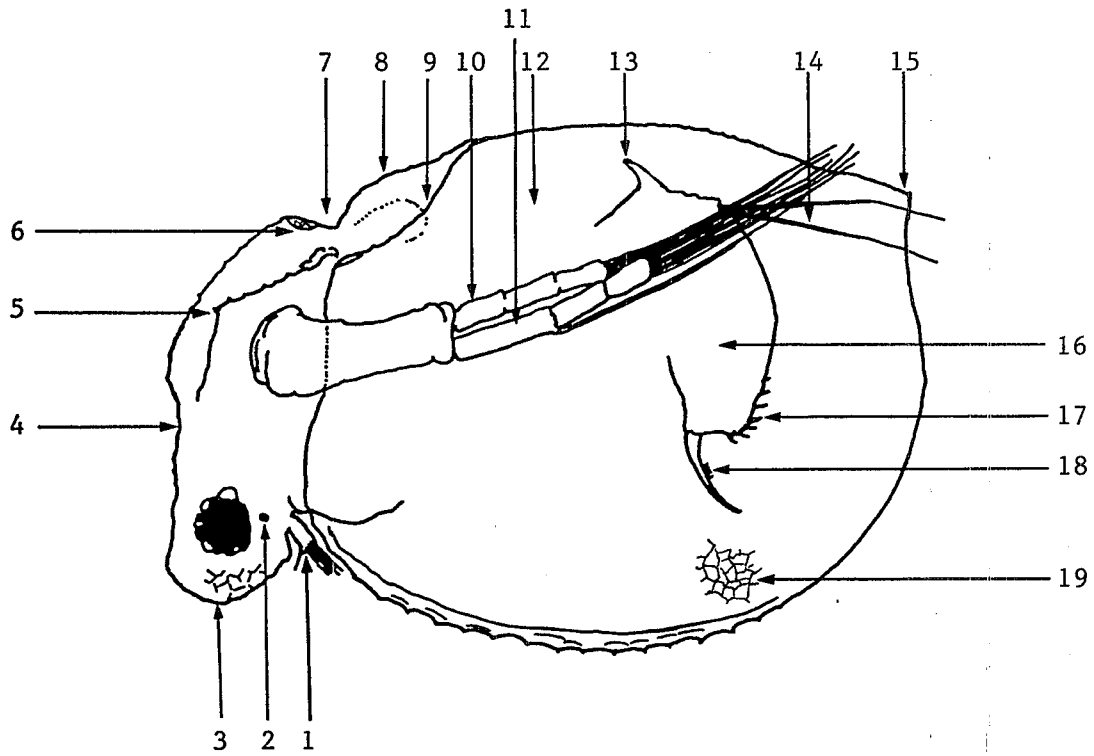
Introduction

Cladocerans from the family Daphniidae are ubiquitous in temperate freshwaters. Numerous field and laboratory studies in the past have focused on Daphnia, which often are abundant in limnetic communities and are large enough to be easily distinguished from other zooplankton. Consequently, techniques have been developed for utilization of Daphnia magna and Daphnia pulex in water quality testing (Buikema et al 1980).

However, because of their relatively large body size, these cladocerans do not reproduce until the 4th to 6th instar after birth (Anderson et al 1937; Anderson and Jenkins 1942) and are best employed in tests with a duration greater than a week.

Ceriodaphnia, daphnids of smaller size and shorter generation times, are also amenable to laboratory culture (Burgess 1967), producing 3-4 broods a week under optimal conditions. Taxonomically, Ceriodaphnia resemble Daphnia (Fig. 1) except that they are more rotund and lack the prominent rostral projection typical of that genus. They exhibit some cyclomorphism, but do not develop the dorsal helmets and long posterior spines often seen in Daphnia. Since 1969, the U.S. Environmental Protection Agency (EPA) Environmental Research Laboratory-Duluth and other EPA and private laboratories have been exploring the suitability of Ceriodaphnia for short-term (7-10 day) toxicity testing.

Initial Ceriodaphnia stock cultures were established during 1969 in the Duluth EPA laboratories with animals obtained from fish ponds at the Newtown Fish Toxicology Station, Newtown, OH (D. Mount 1983 in litt.). The stocks were identified as Ceriodaphnia reticulata. In 1982-83, subtle differences in the appearance of the cultured animals suggested that the stocks comprised more than one species (D. Mount 1983 in litt.). Subsequent microscopic examination in December, 1983, by myself and P. Lewis, of the EPA Environmental Monitoring and Support Laboratory-Cincinnati, Newtown Facility, revealed that their cultures contained two, possibly three, species of Ceriodaphnia. Two were tentatively identified as C. reticulata and C. dubia (or C. affinis because a problem in synonomy exists) but the third was unidentifiable and appeared to have characteristics of both the other two.



1. Antennule with anterior sense hair and terminal aesthetascs (fine sensory hairs)
2. Ocellus
3. Frons
4. Supraocular depression
5. Fornix
6. Fenestra (headpore)
7. Cervical notch
8. Cardiac bulge (heart shown as dotted line beneath)
9. Ecdysial line
10. Exopod of antenna
11. Endopod of antenna
12. Brood chamber
13. Abdominal appendage
14. Abdominal seta
15. Posterodorsal angle
16. Postabdomen
17. Anal denticles
18. Postabdominal claw with a pecten
19. Reticulations

Figure 1--Composite drawing of the lateral aspect of a parthenogenetic female Ceriodaphnia illustrating typical morphological features.

The goals of the study reported herein were: 1. to verify the identification of C. reticulata and C. dubia in the Newtown Fieldsite (and other) EPA cultures, and 2. to try to determine, by taxonomic comparison and by interspecific matings, if the unidentifiable animals were hybrids of the two former species, a morph of one of those species, or a new species.

Conclusions

1. Nearly all Ceriodaphnia cultures at the EPA Newtown Facility and Duluth laboratories, and other cultures derived from them, were C. reticulata or C. dubia.

2. A third Ceriodaphnia found in a few EPA cultures, particularly those from the Athens, GA, laboratory, was a morphological variant of C. dubia, which it resembles almost completely. The morph differs mainly in that the females have a heavy-toothed pecten, somewhat reticulata-like, on the claw. This pecten was reversibly altered to the dubia form with heavy setules when the animals were cultured in reconstituted, rather than well water. The males are indistinguishable from C. dubia males, regardless of culture medium. Specimens of this variant have been found in natural populations of C. dubia west of the Mississippi River (D. Berner 1985, unpublished observations). It is designated in this report as C. dubia, toothed-pecten variety.

3. Experiments attempting to hybridize C. reticulata and C. dubia were inconclusive, although two successful interspecific matings occurred. Taxonomically, these species appear to be closely enough related that males might mistake a female of the other species as their own. To ascertain whether hybridization is possible, more experiments of the kind attempted in this study would have to be carried out. Furthermore, ephippia of successful matings should be gathered and hatched to see if viable populations of hybrids can be produced. Lastly, the morphology of such hybrids should be compared with specimens from field populations in which C. reticulata and C. dubia coexist, to see if hybrid forms occur naturally.

4. Comparison of the EPA C. dubia with N. American and European populations designated C. dubia or C. affinis revealed no significant differences among them. This study therefore supports Johnson's (1956) conclusion that the

two names are synonomous, and that C. dubia Richard 1894 takes precedence over C. affinis Lilljeborg 1901. It is likely, however, that other species of Ceriodaphnia exist that have a heavy, fine-toothed central pecten on the claw similar to that of dubia. Therefore, that character alone should not be used to identify animals found in natural populations.

Materials and Methods

Source of specimens

Most of the Ceriodaphnia examined in this study were from cultures being maintained at the EPA Environmental Monitoring and Support Laboratory, Newtown Facility, OH. Some were from the EPA laboratories in Athens, GA, and Duluth, MN. The following also provided specimens for identification from their cultures, which had originally come fron the Duluth laboratory: P. Dorn, Shell Development, Houston TX; J. Fava, Ecological Analysts, Inc., Sparks, MD; D. R. Folley, N.C. Department of NRCD, Cary, NC; R. Keen, Michigan Technological University, Houghton, MI; A. V. Nebeker, Western Fish Toxicology Station, Corvallis, OR; D. Nimmo, Colorado State University (Region VIII, USEPA), Ft. Collins, CO; J. Owsley, Tennessee Department of Health and Environment, Nashville, TN; R. Rupp, Southern Experimental Streams Facility, NCASI, New Bern, NC; C. N. Scott, Environmental Laboratories, Burlington Research, Inc., Burlington, NC; M. Taylor, Environmental Safety Department, Proctor and Gamble Ivorydale Technical Center, Cincinnati, OH; C. D. Webster, Ohio EPA, Columbus, OH; and J. B. Whittaker, Biological Monitoring, Inc., Blacksburg, VA.

EPA Ceriodaphnia were compared with specimens from the author's personal collection and ones from S. Cooper, Santa Barbara, CA; D. G. Frey, Bloomington, IN; T. Edmonson, Seattle, WA; J. Korstad, Tulsa, OK; W. Murdock, Santa Barbara, CA; W. Nelson, Ft. Collins, CO; and W. Hollwedel, Varel, W. Germany. Specimens were also borrowed from the Kiser collection at the U.S. Natural History Museum (Smithsonian), Washington, DC; the British Museum (Natural History), London; and the Lilljeborg Collection, Uppsala, Sweden.

Preservation of specimens

All museum specimens were preserved in alcohol or mounted on slides. Those from laboratory cultures and personal field collections were in formaldehyde solution or formaldehyde-sucrose (Haney and Hall 1973). Fixation often distorts Ceriodaphnia, especially those cultured in reconstituted water, which have particularly soft exoskeletons. To avoid 'ballooning' of the carapace and retraction of the postabdomen up against the body during fixation of live specimens for this study, animals were first concentrated in a small volume of water. Ninety-five percent ethanol saturated with sucrose (table sugar) was gradually added until the animals ceased swimming and relaxed so that their postabdomens could be seen at 30X under a dissecting microscope. A proportionate amount of 37-40% formaldehyde solution saturated with sucrose was then added to achieve a final 2-4% formaldehyde concentration.

Microscopy

Specimens to be examined by light microscopy (LM) were transferred to 1:1 glycerol-water solution in a deep depression slide and allowed to clear for an hour or more. Small chambers for microscopy were made of two parallel strips of plastic coverslip enclosing a small volume of 100% glycerol. Individual animals were moved into this with an etched tungsten wire loop. Sometimes they were propped into position in the chamber by snips of strands from a cotton ball. Frequently the swimming antennae of animals to be viewed laterally were first dissected away with tungsten wire needles; this made it easier to orient them and view the head and antennules. No coverslips were used on whole mounts in order to avoid distortion. Appendages to be examined in detail, such as swimming antennae and postabdomens, were first dissected free of the body in 1:1 glycerol-water, then mounted under a coverslip in a drop of 100% glycerol or CMC9-AF water-miscible medium (Masters Chemical Company).

An Olympus BH-2 microscope equipped with phase-contrast optics and an Olympus LB drawing tube (camera lucida) was used for observations and drawings. Most structures in whole mounts could be visualized best with direct illumination and the condenser diaphram stopped down to provide oblique light. Phase-contrast was used only for appendages mounted under coverslips. Whole animals were drawn with direct illumination; postabdomens were drawn with both phase-contrast and direct illumination. Measurements were made with ocular and stage micrometers and a millimeter rule on the drawing surface.

Specimens for scanning electron microscopy (SEM) were dehydrated in two changes of 30, 50, 80, and 95% ethanol for at least 30 minutes at each step. Final dehydration was in 2-3 changes of 100% ethanol for several hours or overnight. Specimens were transferred to small, capped carriers made from #10 plankton netting and size 00 Beem capsules (Ernest F. Fullam, Inc.), and critical point dried from CO_2 in a Tousimis drier. An eyelash mounted on a probe was used to mount the animals on double-stick tape affixed to a stub. Specimens were spatter coated with gold-palladium. Scans were made with an "Ameray" AMR model 1000 microscope and photographed with Polaroid 55 negative-positive film.

Most of the specimens used for LM drawings and SEM in this study were from cultures at the Newtown Facility. A few were from cultures sent to the author directly from EPA laboratories at Duluth, MN and Athens, GA.

Mating experiments

Gamogenetic Ceriodaphnia (males, ephippial females, and sexual females in the sterile, pre-ephippial instar) can, with some difficulty, be isolated from living cultures. Sexual females are recognizable in the sterile, pre-ephippial instar because they have a dark egg mass in only one ovary and the dorsal carapace is compressed laterally. In late sterile instar and unmated ephippial females the clear or orangish lateral bulges and greyish borders of the ephippium are visible in addition to the single, dark ovary. A single, dark egg is evident in the ephippium of a sexual female who has already mated (Fig. 10.2). Males resemble juvenile females, especially in earlier instars. More mature males can be identified by their posteriorally tapered bodies, extended antennules and claspers, reddish-pink color, and restless swimming behavior.

Gamogenetic individuals were sometimes located by examining cultures in fingerbowls with a binocular microscope and direct illumination. More often, glass beakers containing cultures were placed directly on a light table and examined with a head or ring-mounted magnifier. Individual animals were removed with a largemouth pipet and isolated in small Stender dishes. Male C. reticulata were particularly difficult to identify because of their small size and insignificant antennules. An attempt to isolate them was made by pouring cultures through a fine sieve, resuspending the animals in a small amount of medium, then anaesthetizing them with CO_2 (Club Soda) in order to sort them under a binocular microscope. This technique yielded a number of males. How-

ever, although all the animals resumed swimming following their return to culture water, only females survived. The males died within 12 hours and this technique had to be abandoned. Thus very few male C. reticulata were available for use in this study.

Animals to be mated were kept in pond or culture water and fed one or two drops of cerophyll-yeast-trout chow medium (formula supplied verbally to the Newtown Facility by the EPA Environmental Research Laboratory-Duluth, T. Norberg 1985 personal communication). The planned procedure for these experiments was to place a mature male with one or more sexual females of the same species and to make observations at periodic intervals until one or more females had an egg in her ephippium, evidence of succesful mating (see Results, below). The male would then be isolated with sexual females of another species and observed to see if mating occurred. If none was seen after two or three days, the male would again be placed with sexual females of his own species as a control to see if he was still fertile. Because of culture conditions, this procedure could not be carried out fully (see Results). The males and females used in these experiments were preserved in formalin-sucrose for later examination.

A few of these mating experiments were carried out in the author's laboratory; the rest were done during a week's stay at the Newtown Facility.

Results

Taxonomy

The Ceriodaphnia examined in this study fell into three groups. Two could clearly be classified as C. reticulata and C. dubia. The third, at first thought to be a hybrid of the first two, or even a new species, has been determined to be a variety of C. dubia. Descriptions of the two known species and the new variety are presented separately and then their similarities and differences are considered.

For those unfamiliar with cladoceran taxonomy, Fig. 1 presents most of the morphological characters used in this study. A general description of Ceriodaphnia morphology is given in Appendix A. In most of the drawings

accompanying the descriptions, the swimming antennae, which vary little in detail, have been omitted for the sake of simplification.

1. Ceriodaphnia reticulata

Parthenogenetic female. Adult length, to 0.77 mm. Height, from 0.6 to 0.8 times length, depending upon maturity and number of eggs in brood chamber. Shape, oval to almost round (Fig. 2.1, 4.4-.5, 5.1). Head, quite depressed, with the frons at or below the level of the ventral carapace margin. A distinct supraoptical depression on the anterior head surface bordered by arched lateral margins. Ventral margin of head curved, with little or no angle anterior to antennule but sometimes with protruded edges of reticulations that look like tiny spines (Fig. 4.2, 4.3). Cervical notch broad and shallow with oval fenestra on anterior margin. Dorsal margin of carapace arched, terminating in a distinct point at the posterodorsal angle. Lateral bulge of brood chamber extends anteriorally into posterior headshield (Fig. 4.4, 5.1) and may rise above the mid-dorsal line (Fig. 4.4). Individuals with many young may be more rounded, shorter, and have a more distinct, lower posterodorsal point than females with fewer eggs (Fig. 4.5). Antero- and posteroventral carapace margins evenly and equally curved. Posterior carapace margin sometimes with one or more tubercles that mark pores connected to glands. Fornix has a low arch, sometimes with a small spine on the fold dorsal to the antenna. Reticulations on carapace small in area, with distinct but not prominent edges. Head and headshield usually smooth (in EPA cultures) with a single, raised edge running from the anterior fornix ventrally to the base of the antennule.

Eye fairly large, nearly filling anterior-ventral portion of head. Ocellus small and triangular, located close to base of antennule. Antennule cylindrical and short, not extending beyond line of margin of head. Nine aesthetascs, about equal in length to length of antennule. Anterior sensory seta arising from a small peduncle near apex of antennule. Antenna is of typical morphology, with setae that do not extend as far back as the posterior carapace margin (Fig. 2.1, 5.1).

Postabdomen (Fig. 3.1) long, narrow (length about 3X width) and gently tapered with a slight, mid-dorsal inflection. Abdominal process present, sometimes long and tapered, separated from abdominal setae by three dorsal rows of long, fine setae or hairs. Patches of short setae and very fine hairs

on lateral surface anterior to abdominal setae. Anal denticles about 8 in number, long, fine, and recurved, decreasing in length proximally except for most distal 1 or 2, which are short and straight (Fig, 3.1, 5.2). Two rows of fine setules on lateral surface adjacent to anal denticles; proximally, these break up into scalloped clusters, with heavier spinules adjacent to the dorsal inflexion. Postabdominal claw long, slightly recurved, with three distinct divisions of setules and denticles on the lateral surface (Fig. 3.2, 5.2). Setules of most proximal set number about 16, are short and distinctly heavier than ones in the distal group. Denticles in the middle set form a pecten of 2-8 heavy, sharp, triangular teeth that are the outstanding characteristic of this species. They are separated by distinct gaps from the sets to either side. The distal group of fine, short setules runs nearly to the tip of the claw, terminating in one heavier spinule.

Juvenile females resemble adults except that the dorsal margin of the carapace is flattened and the posterodorsal point is higher (Fig. 4.1-4.3). The pecten on the postabdominal claw is visible at 200X magnification in intact specimens of the second instar juvenile (Fig 4.2).

Gamogenetic female (Fig. 2.2, 5.3). Length, about 0.73mm. Height, about 0.76 times length. Shape rounded, flattened dorsally along top of ephippium. Lower borders of ephippium forming a rounded curve or a broad V. Ephippium exhibits three distinct regions: a flattened border region lacking cellular outlines, a raised, semicircular region of deep polygonal cells having slightly domed surfaces, and the dorsal locule, which is covered with small, circular bumps (Fig. 5.3). These become more prominent as ecdysis (moulting) approches. The ephippial surface is decorated with extremely short, stubby hairs (Fig. 5.4). A single, dark egg forms (in either ovary) in the sexual female; it moves into the brood chamber only as a consequence of mating. Color of the ephippium is usually reddish orange.

Other characteristics are as in the parthenogenetic female.

Male. Length, about 0.58mm. Height, about 0.55 times length. Shape, elongate oval, flattened dorsally and ventrally (Fig. 2.3). Head, larger in proportion to body than female, and not as fully depressed, with a distinct dorsal fenestra and supraocular depression. Antennule only slightly longer than that of female, with short aesthetascs and a very short, straight terminal male seta, equal or shorter in length than the body of the antennule.

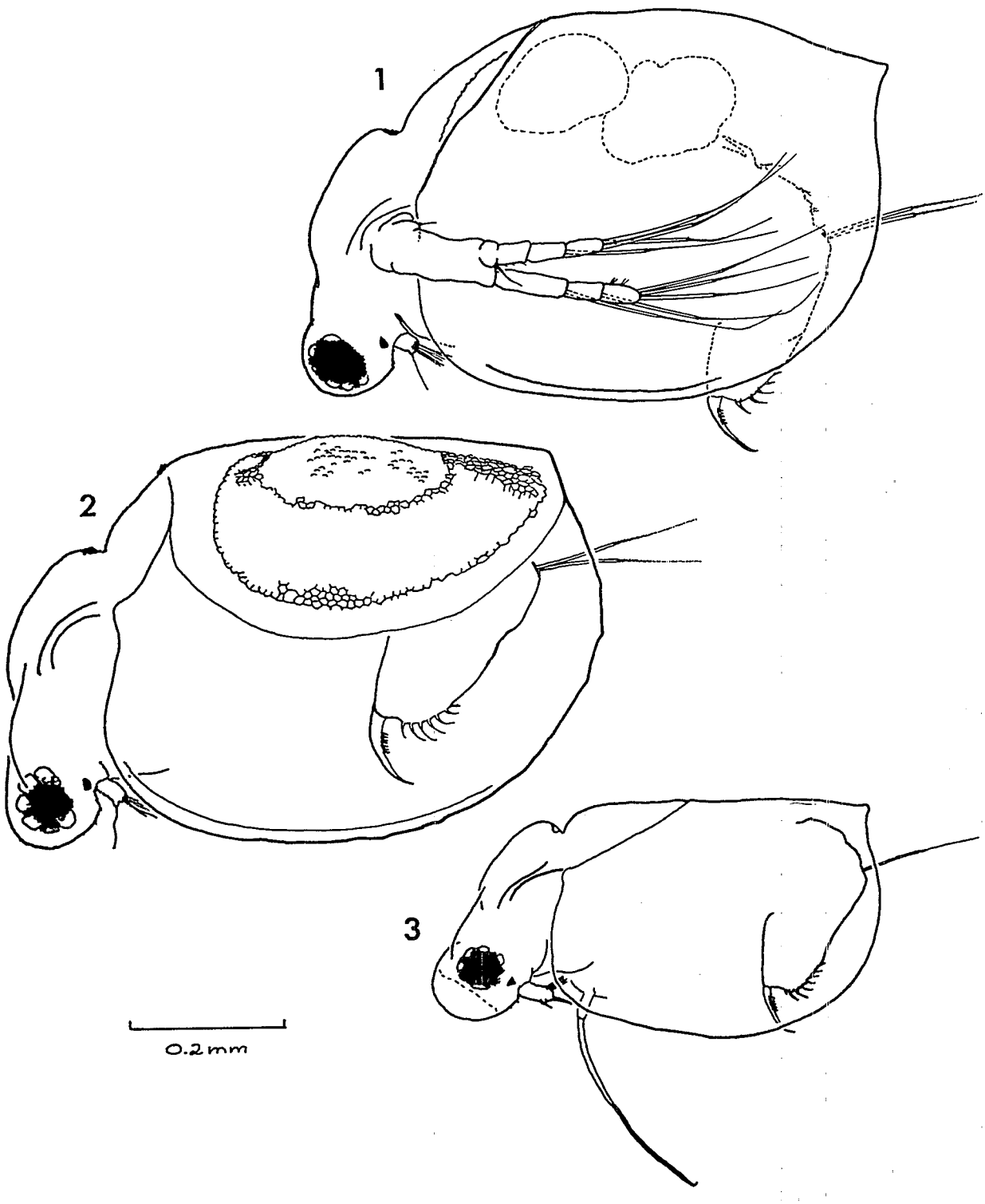


Figure 2--Ceriodaphnia reticulata: 1. parthenogenetic female; 2. sexual (ephippial) female; 3. male.

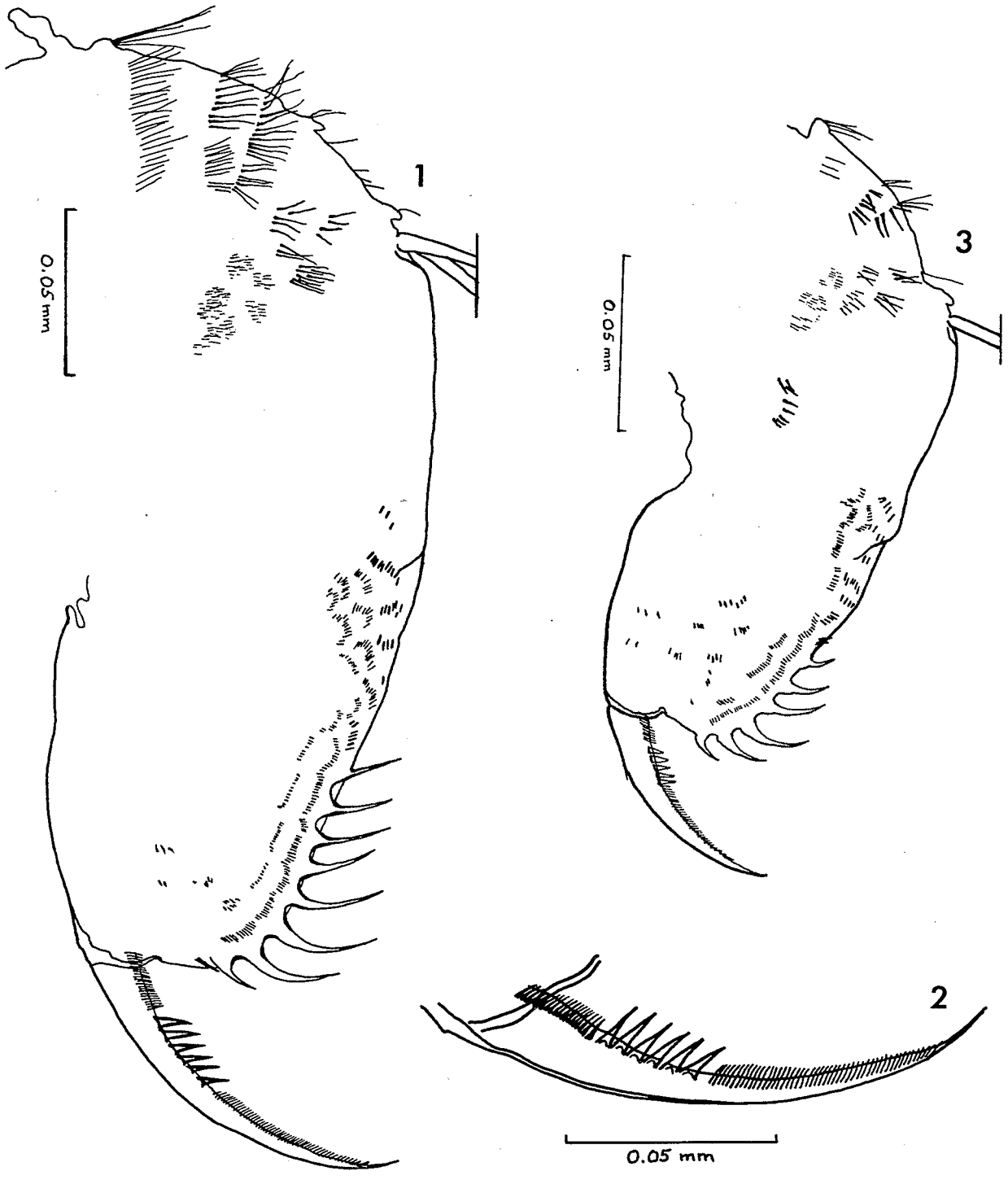


Figure 3--Ceriodaphnia reticulata postabdomens: 1. parthenogenetic female; 2. detail of claw of (1.); 3. male.

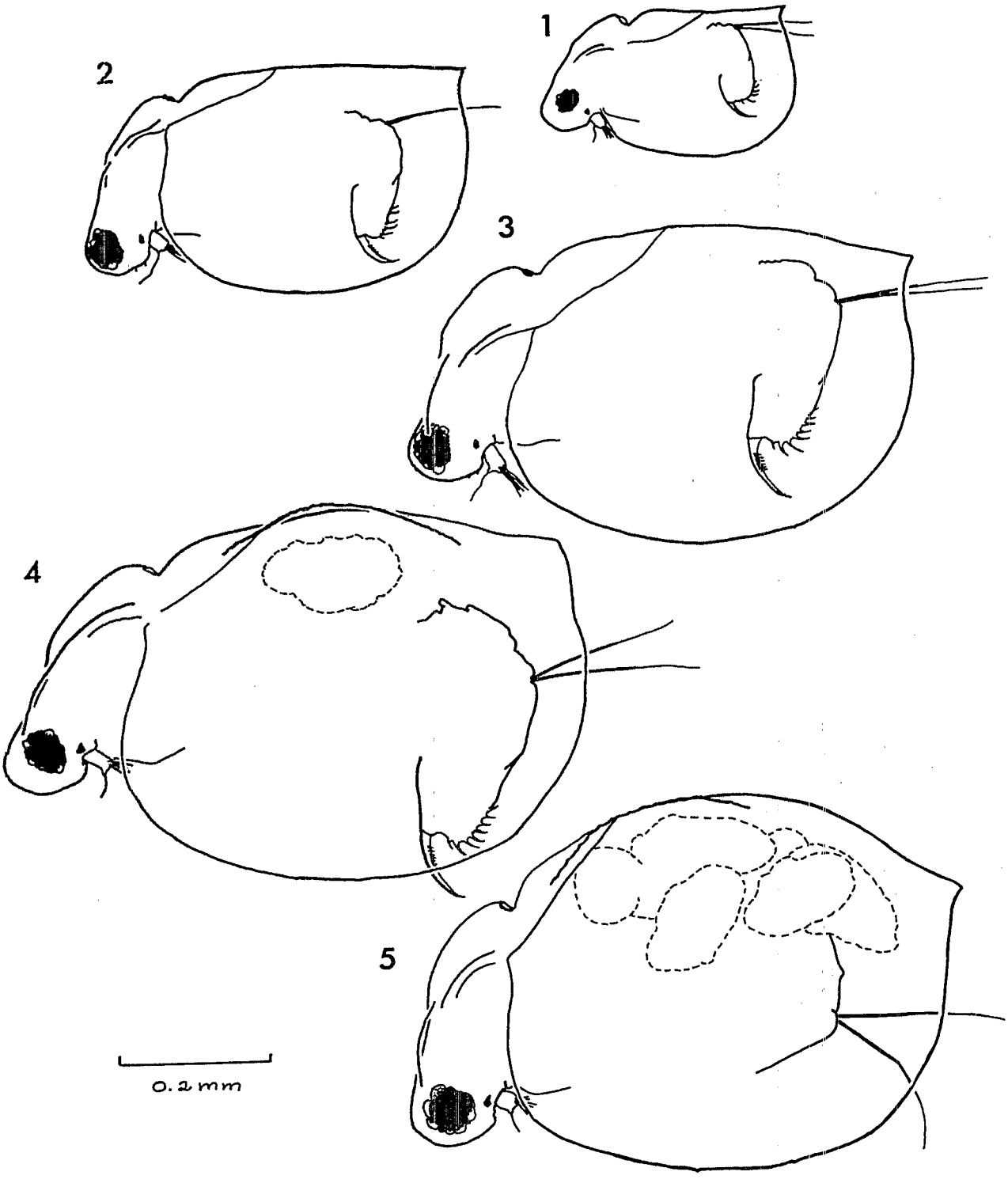


Figure 4--Shape changes during growth of Ceriodaphnia reticulata parthenogenetic females: 1.-3. juveniles; 4., 5. adults.

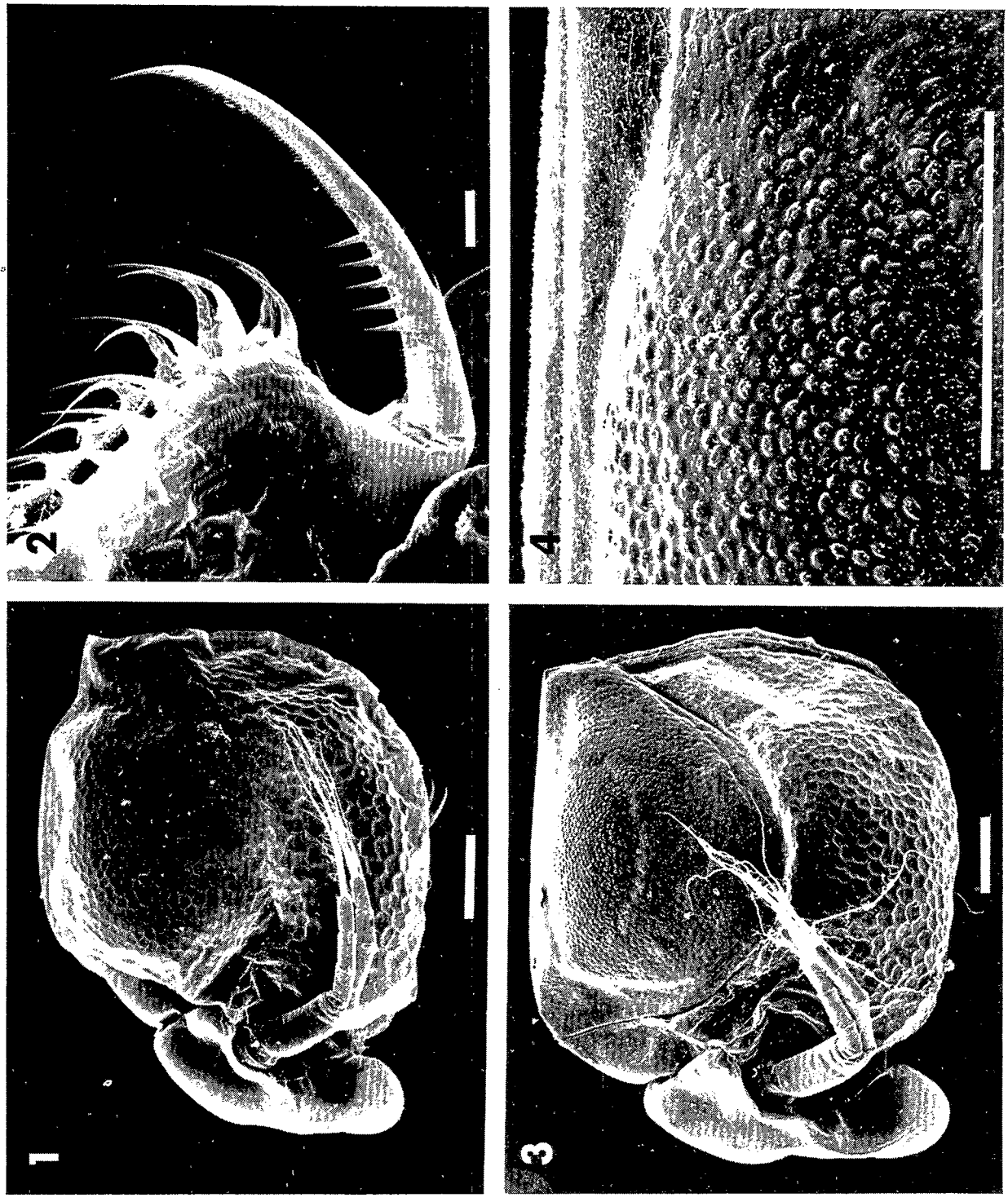


Figure 5--SEM of Ceriodaphnia reticulata: 1. parthenogenetic female; 2. detail of postabdominal claw; 3. ephippial female; 4. detail of ephippial surface. Bar measure: in 2 = 0.01 mm; in others = 0.1 mm.

First thoracic appendage with a typical clasper that terminates in a right-angled hook. Postabdominal size proportions and characteristics as in the female but lacking an abdominal process. Termination of the sperm duct laterally near distal denticles was not observed in this study.

2. Ceriodaphnia dubia

Parthenogenetic female. Adult length, to 0.88mm. Height, about 0.6 times length. Shape, roundish oval (Fig. 6.1, 8.4, 8.5). Head not fully depressed, with frons not as low as ventral margin of carapace. Anterior surface of head with distinct supraorbital depression having arched lateral borders. Ventral margin of head smoothly curved with a slight protuberance or distinct angle anterior to antennule; raised edges of reticulations in this region may appear from lateral aspect as short spines (Fig. 6.1, 8.3). Cervical notch broad with a distinct oval fenestra on the anterior surface (Fig. 9.2). Headshield flat on either side of cardiac bulge, appearing shelf-like in living specimens (Fig. 6.1, 9.1). Dorsal margin of carapace broadly arched, with a medial ridge that is apparent in living specimens viewed posteriorally. Posterodorsal angle blunt, sometimes broadly pointed in mature individuals, located high above the body axis. Anteroventral carapace margin more broadly curved than posteroventral margin, which arches dorsally in a circular curve. Posteror margin sometimes with tubercles at the orifice of pores leading to glands. Fornix with a low arch, not (in EPA cultures) expanded laterally into a wing. Reticulations evenly sized on carapace, sometimes with heavy edges, and with dotted surfaces (Fig. 9.2). Headshield and head usually reticulated, but sometimes smooth, usually with a distinct row of elongate polygons extending from the fornix to the antennule.

Eye large, nearly filling the anterior-ventral portion of the head, with pigment mostly obscuring the crystalline lenses. Ocellus small, roundish, located 1/3 to 1/2 the distance from antennule to eye. Antennule cylindrical and long, extending beyond the line of the head. Aesthetascs 9, as long as antennule. Anterior sensory seta long, arising from a distinct peduncle 1/3 distance above apex of antennule. Antenna of usual character, with terminal setae reaching nearly to posterior margin of carapace (Fig. 6.1).

Postabdomen (Fig. 7.1) moderately long and wide (about 2X as long as wide), tapered, with a slight mid-point inflexion. Abdominal process usually moderate in size (Fig. 7.1), sometimes long (Fig. 8.5). Three rows of fine

dorsal setae between abdominal process and abdominal setae; small patches of short hairs on lateral surface anterior to the latter. Anal denticles 7-8 in number, most distal two short, others longer and diminishing in size proximally. Denticles with a stout base, tapered, and only slightly recurved. One row of setules on lateral surface at base of denticles, continuing proximally to the mid-point inflexion as two to three rows of crescent-shaped clusters, with heavier spinules along the dorsal margin. Postabdominal claw moderately recurved, with three subdivisions of the lateral setules. Setules of proximal group short and slightly lighter in weight than those of distal set. Those of middle set number from 18-24 and are heavier, forming a fine comb or pecten; height of pecten varies among individuals and populations, making it more or less prominent. It is not visible at 200X magnification in intact specimens.

Juvenile females have the same characters as the adult except that their shape is more elongate and the dorsal carapace margin is flattened and even slightly depressed in the first two instars (Fig. 8.1-8.3).

Gamogenetic female. (Fig. 6.2, 9.3). Length, about 0.71mm. Height, about 0.72 times length. Shape, rounded, flattened dorsally along margin of ephippium. Head quite depressed, with frons at level of ventral carapace margin. Ephippium of usual shape. Marginal cells of ephippium with flat surfaces, semicircular band of deep, polygonal cells slightly rounded on top. Surface of locule nearly smooth in early ephippium (Fig. 9.4), becoming irregularly slightly bumpy in mature ephippium (cf. Fig 13.4). Short, stubby hairs decorate the ephippial surface (Fig. 9.4). Ephippium has a greyish coloration, sometimes with an orangeish locule, depending on culture conditions. Single ephippial egg develops in ovary and moves to brood chamber following mating.

Male. (Fig. 6.3). Length, to 0.66mm. Height, about 0.57 times length. Shape, quadrangular. Head, noticeably large, not fully depressed, with a supraorbital depression and slightly inflated appearance. Cervical notch broad, with a distinct fenestra on the anterior border. Eye larger than in a female of comparable size. Antennule long and cylindrical with terminal male seta 1.5 times length of antennule, terminating in a curved hook. Clasper on second thoracic appendage long and thin, curving to a small terminal hook. Postabdomen (Fig. 7.3) with denticulation and setulation like that of the female, but somewhat narrower, proportionally, and lacking an abdominal appendage.

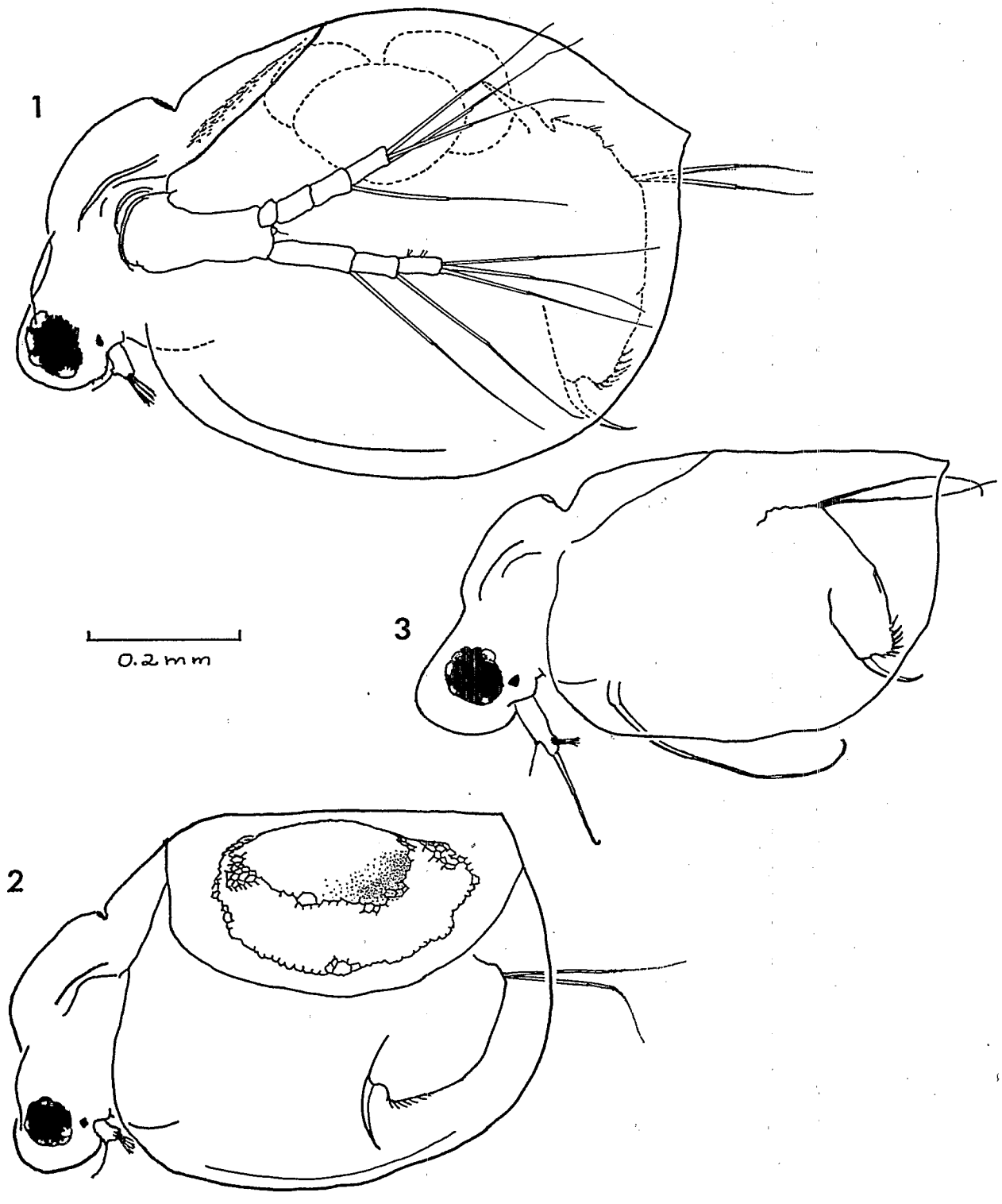


Figure 6--Ceriodaphnia dubia: 1. parthenogenetic female; 2. sexual (ephippial) female; 3. male.

Figure 7--Ceriodaphnia dubia postabdomens: 1. parthenogenetic female; 2. detail of claw of (1.); 3. male.

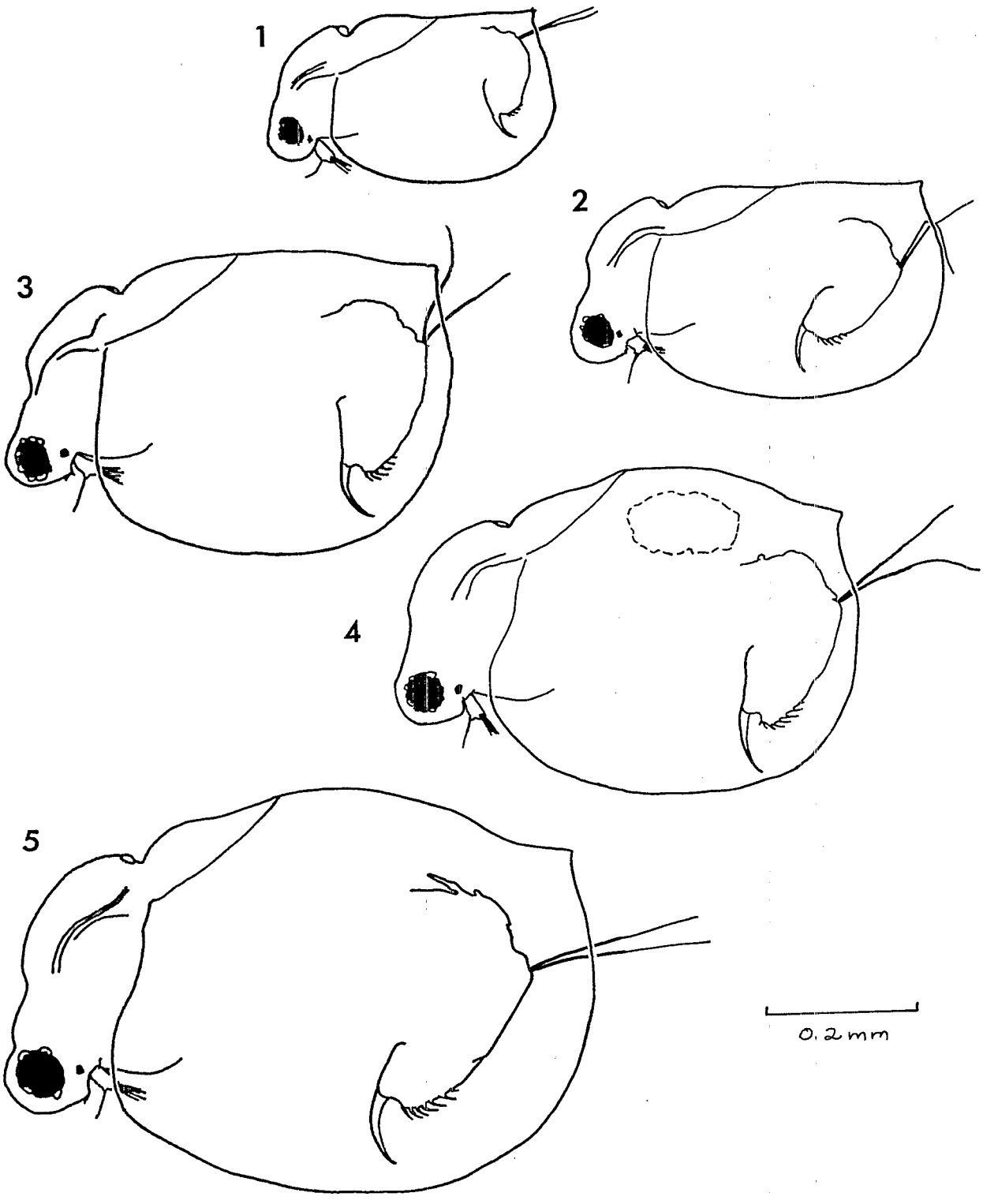


Figure 8--Shape changes during growth of Ceriodaphnia dubia parthenogenetic females: 1.-3. juveniles; 4., 5. adults.

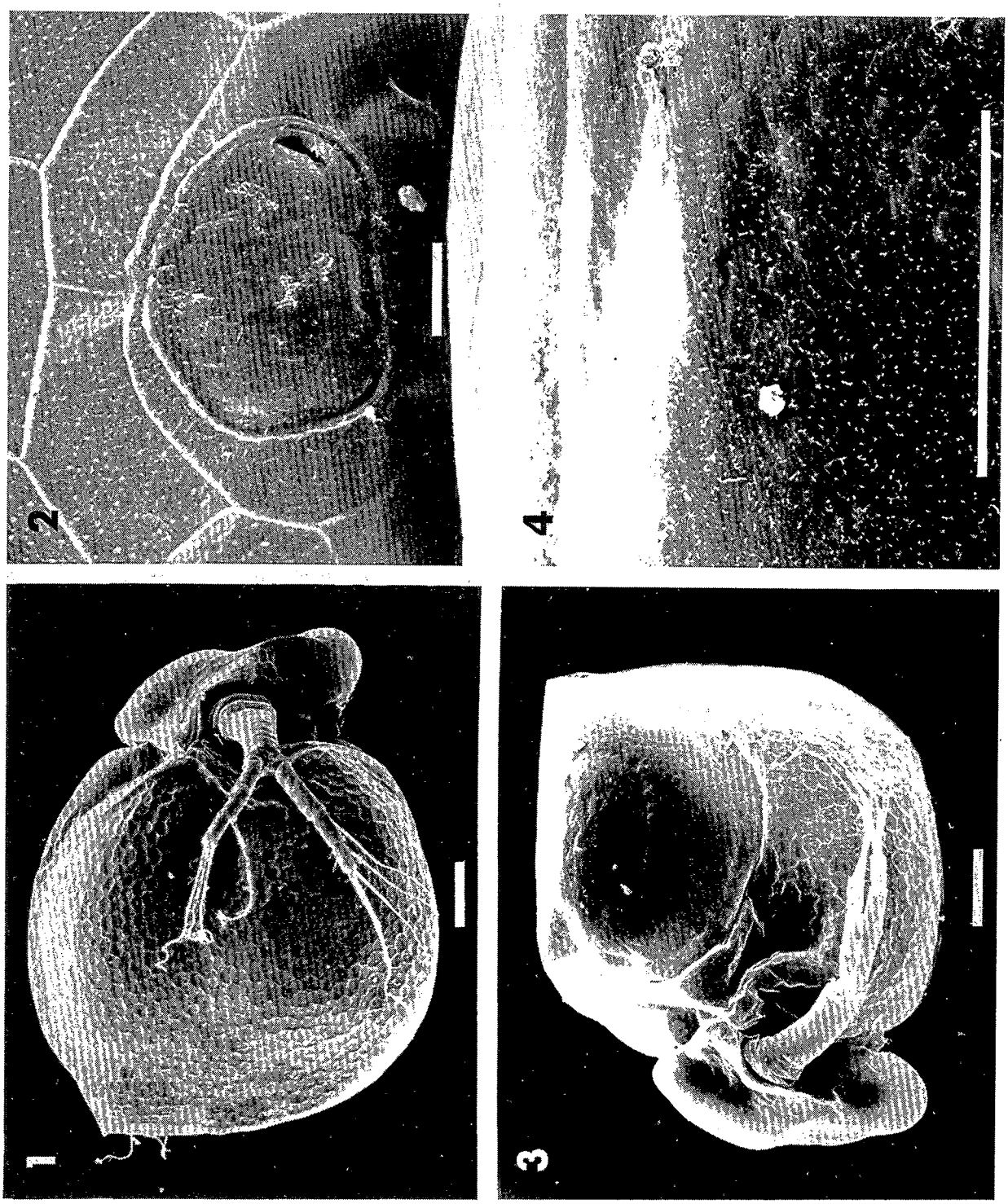


Figure 9--SEM of Ceriodaphnia dubia: 1. parthenogenetic female; 2. detail of dorsal fenestra; 3. ephippial female; 4. detail of ephippial surface. Bar measure: in 2 = 0.01 mm; in others = 0.1 mm.

3. Ceriodaphnia dubia, toothed-pecten variety

Parthenogenetic female. Length, to 0.90 mm. Height, about 0.7 times length. Shape and other characteristics of adult and juveniles like those of C. dubia (Fig 10.1, 12.1-.5), with the following exceptions:

Edges of polygons on carapace and head heavy, so that reticulations show clearly (Fig. 13.1).

Postabdominal claw with either of two forms of central pecten, depending on culture medium. In one form, pecten is a comb of heavy, fine setules slightly longer than adjacent groups; claw is indistinguishable from that of C. dubia. In the second form, setules have been transformed to 7-14, close-set, ovately tapered denticles that appear (in SEM) to be somewhat flexible (Fig. 11.1, 11.2, 13.2).

Gamogenetic female. Length, to 0.9mm. Height, about 0.77 times length. Except for heavier reticulation of the carapace (Fig. 13.3), and possible presence of a heavier pecten on the claw (Fig. 10.2) characteristics are the same as for C. dubia, including ornamentation of the ephippial surface (Fig. 13.4).

Male. Length, to 0.66mm. Height, about 0.56 times length. In all its characteristics, male cannot be distinguished from a male C. dubia (Fig. 10.3). Postabdominal claw always has a central, comb-like pecten, even in cultures where females have the heavier form of pecten.

4. Comparison of EPA Ceriodaphnia with other populations and descriptions

The EPA C. reticulata did not differ noticeably from populations collected in Wisconsin, New York, and Massachusetts, and also were similar to two northern European populations that were examined. None of the males from these populations had an antennule as long as shown by Lilljeborg (1901) in his classical description. All N. American gamogenetic females had ephippia with circular bumps and tiny spines but the ephippia of European specimens lacked such bumps and looked more like C. dubia ephippia. In all of the specimens examined, the denticles of the middle claw pecten numbered from 2 to 8; they were straight-edged, heavy, sharp, and separated slightly from each other and from the spinules to either side.

The EPA C. dubia, in general, were similar to populations collected in California, Colorado, Louisiana, Mississippi, and Oklahoma, and to ones from England, and N. Germany. Although no morphometric analysis was done, no

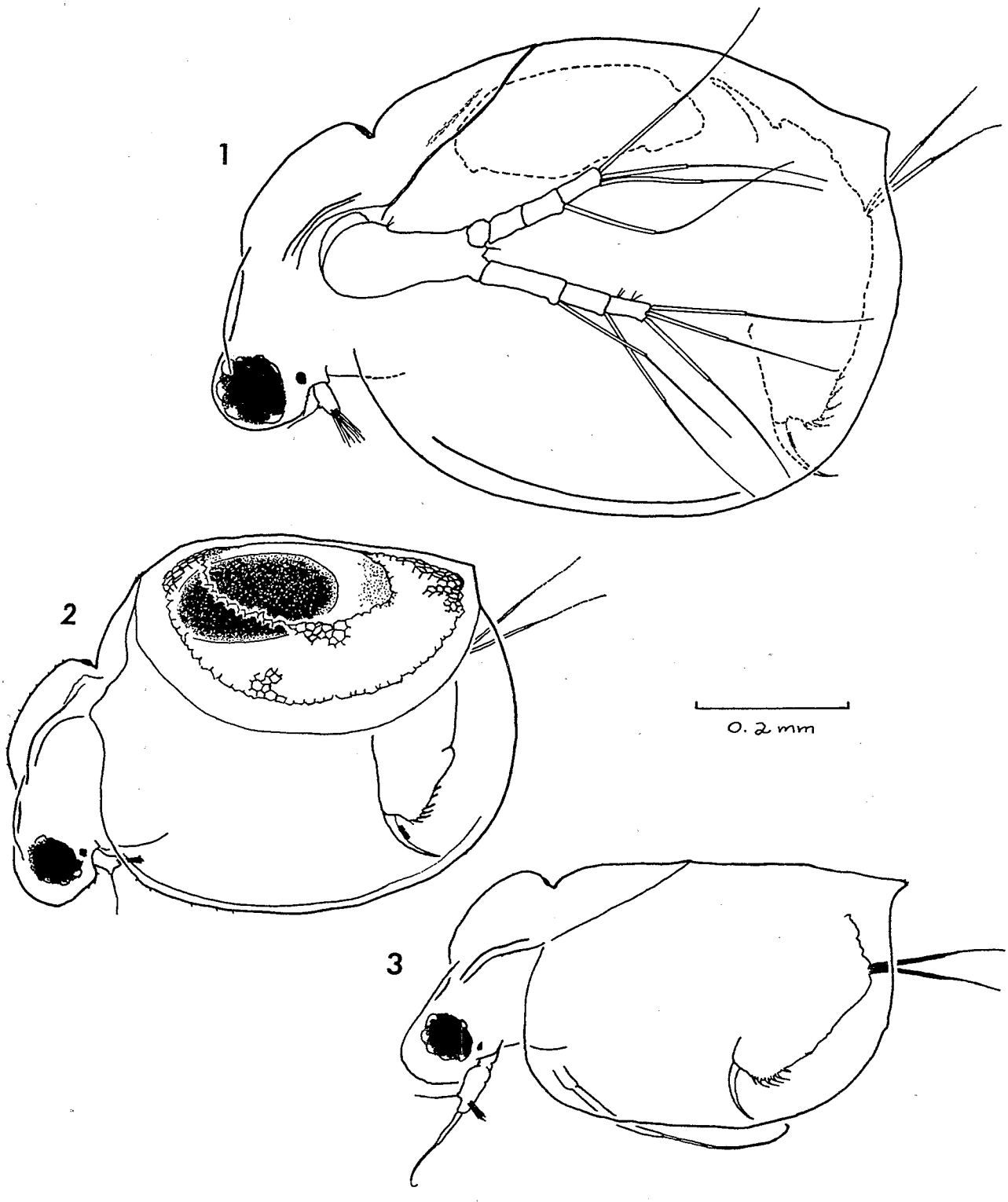


Figure 10--Ceriodaphnia dubia, toothed-pecten variety: 1. parthenogenetic female; 2. sexual (ephippial) female; 3. male.

Figure 11--Ceriodaphnia dubia, toothed-pecten variety postabdomens: 1. parthenogenetic female; 2. detail of female claw; 3. male; 4. detail of claw of (3.).

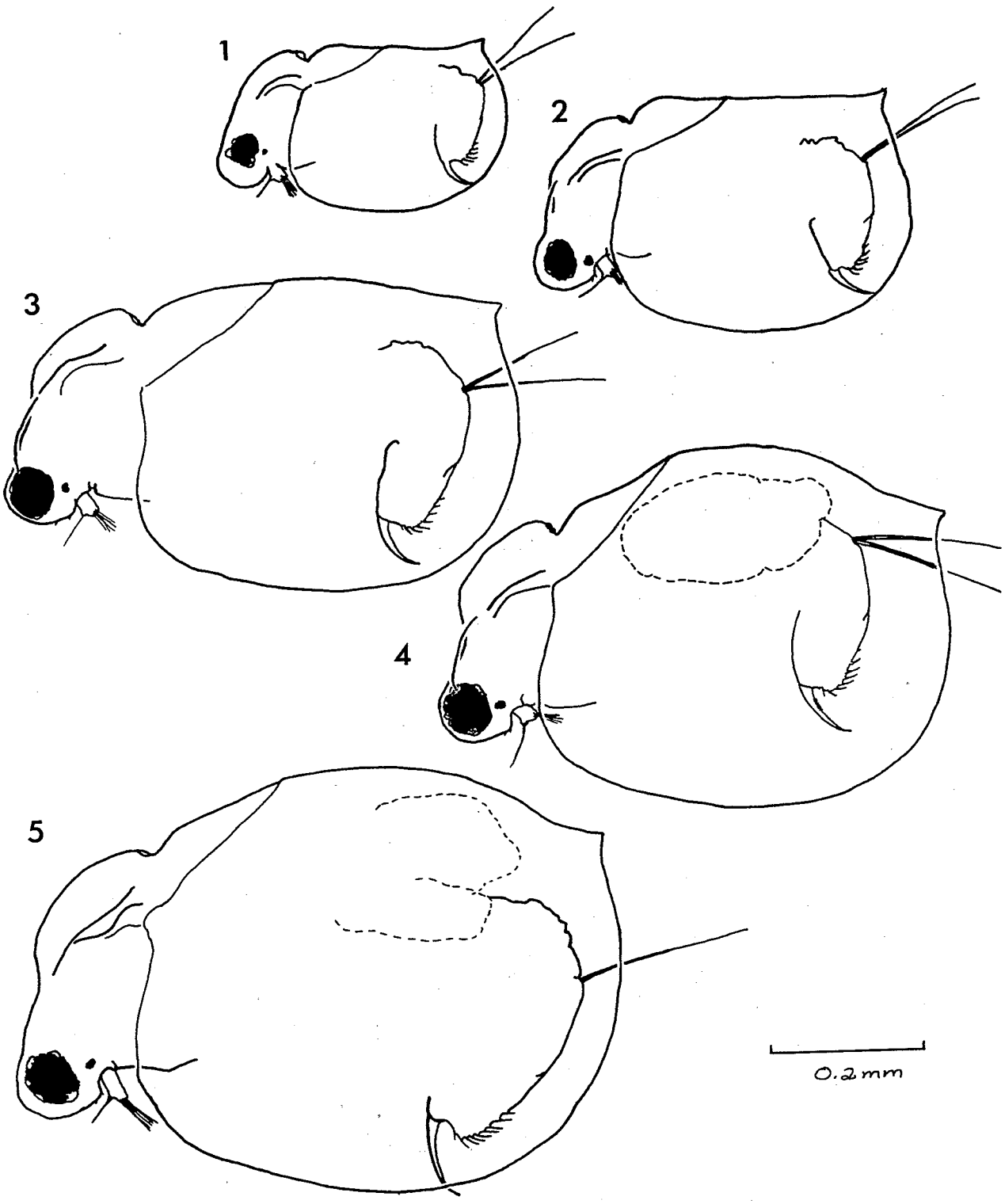


Figure 12--Shape changes during growth of Ceriodaphnia dubia, toothed-pecten variety parthenogenetic females: 1.-3. juveniles; 4., 5. adults.

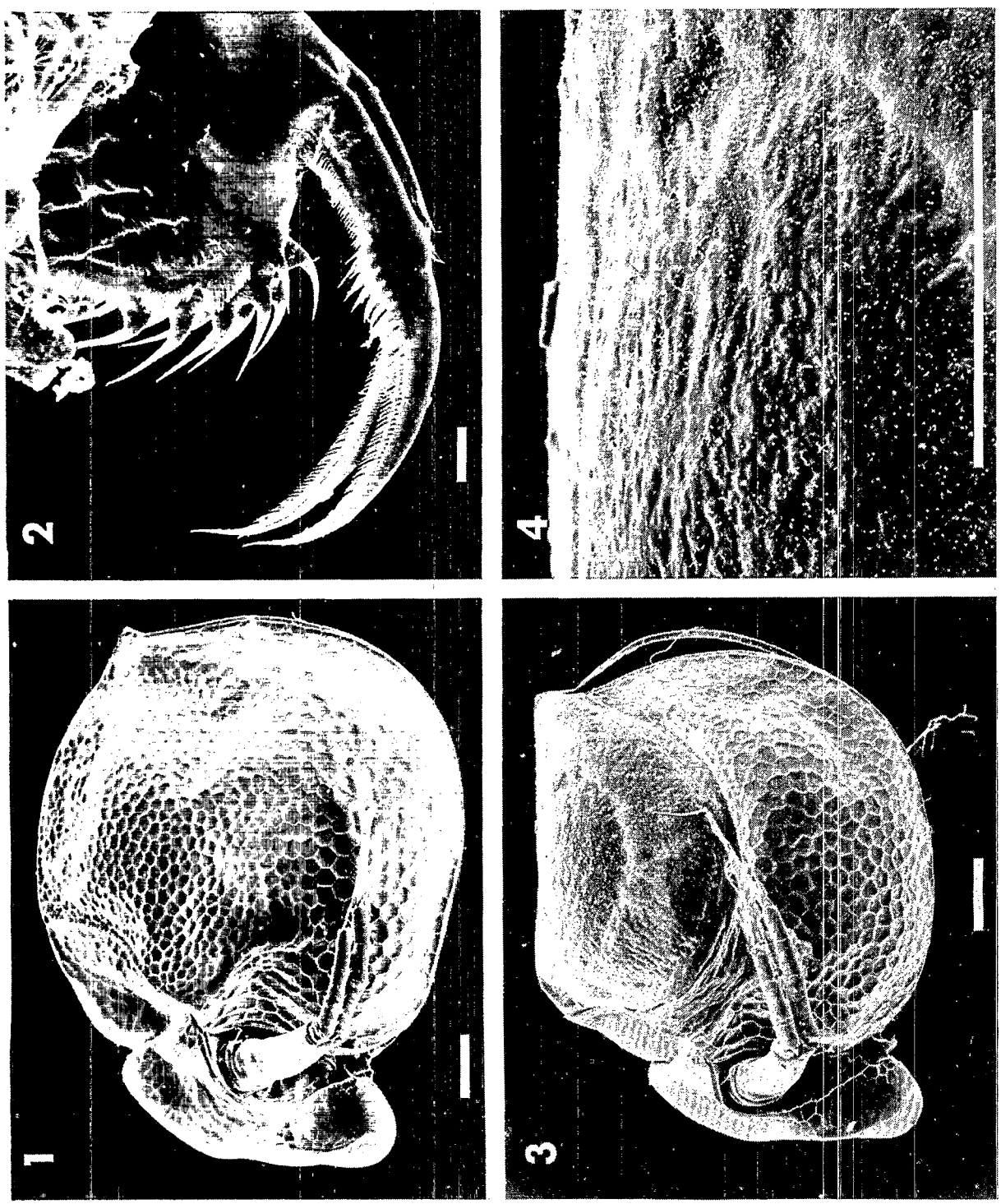


Figure 13--SEM of Ceriodaphnia dubia, toothed-pecten variety: 1. parthenogenetic female; 2. detail of postabdominal claw; 3. ephippial female; 4. detail of ephippial surface. Bar measure: in 2 = 0.01 mm; in others = 0.1 mm.

obvious differences were observed among the EPA animals, Lilljeborg's (1901) type population of C. affinis, and specimens labeled either 'affinis' or 'dubia' in the British Museum (Natural History) collection. The only significant way in which the EPA C. dubia differed from other populations was that they did not exhibit the degree of polymorphism (widened, spined fornices and heavily reticulated morphs) that often occurs in natural populations (Johnson 1956 and this author, unpublished observations).

There are no published descriptions of a Ceriodaphnia with a pecten like that of the C. dubia variety described herein. It does occur in nature, however, and has been found in populations of C. dubia from Colorado, Oklahoma, Oregon, and California (D. Berner, unpublished observations).

Mating Experiments

The Ceriodaphnia in the EPA Newtown Facility responded differentially to the culture conditions of reconstituted water and a trout chow-yeast-Cerophyl food mixture. C. reticulata cultures steadily produced a small percentage of sexual females, but very few males. The C. dubia cultures, on the other hand, rarely had sexual females but always produced some males. C. cf. dubia cultures, which had recently arrived from the Athens, GA, EPA facility, had few gamogenetic individuals of either sex. Therefore, the original plan of using adult males for consecutive intra- and interspecific mating could not be carried out because of lack of sufficient gamogenetic individuals.

Nevertheless, enough C. dubia males were found to attempt matings with gamogenetic females: 20 with C. dubia and 17 with C. reticulata. Of the former, 3 were successful; of the latter, 2 were successsful. A successful mating was judged to have occurred when a dark resting egg was observed in the ephippium. This criterion was based on two observations. Firstly, no egg was subsequently observed in the ephippium of a female who had been carrying the egg in her ovary at the time she was isolated from males. When these females moulted, empty (sterile) ephippia were released. Secondly, one pair of C. dubia in a culture was discovered mating. They separated after several minutes and were isolated into individual dishes. When the female was observed twenty minutes later, the egg had already moved into the ephippial chamber. It was concluded that an egg is found in the ephippium only as a consequence of copulation.

In a few cases, matings were unsuccessful because animals died, perhaps because of poor nutrition. In six cases, the males were probably not mature. Two C. dubia females turned out to be parthenogenetic females bearing single eggs rather than sexual females in the pre-ephippial instar. However, in most of the unsuccessful pairings the selected females had well-developed ephippia that were moulted within 24hr of isolation with a male. Four of the successful matings occurred with females that moulted from the sterile instar to the ephippial instar while with the male. One female that had just moulted from the sterile instar before isolation with a male also copulated. It thus appears that there is only a relatively brief period of time following the sterile instar moult during which copulation occurs.

Discussion and Recommendations

The EPA and N. American C. reticulata observed in this study do not appear to differ significantly from northern European specimens except that the surface of the ephippium has distinct bumps, or broad tubercles, in the former but not in the latter. This may be a matter of interpopulational difference rather than an indication of interspecific difference. Therefore, it is concluded that the species in some of the cultures at the EPA Newtown Facility and the EPA Environmental Research Laboratory-Duluth was Ceriodaphnia reticulata (Jurine 1820).

The second species being cultured in these two laboratories and found in most cultures derived from the Duluth cultures was identified as Ceriodaphnia dubia Richard 1894. This study supports the view of Johnson (1956) that C. affinis and C. dubia are synonomous, with the latter name taking precedence. Johnson had worked extensively with European populations of C. affinis Lilljeborg 1901 prior to examining specimens from Richard's type locale, and gave good arguments in support of his opinion. This synonomy was accepted by Scourfield and Harding (1958). Nevertheless, the name affinis has come into general usage because many temperate latitude investigators use Lilljeborg's (1901) text for identification. The identification of this species in N. America is further confused by the fact that Brooks (1959) commonly used key does not describe it under either synonym. It perhaps is frequently mis-identified as

C. quadrangula. It should be cautioned that probably not all Ceriodaphnia with a long, finely setulated middle pecten on the claw are C. dubia: at least two populations have been found along the NE American coast that resemble C. dubia in this respect but have markedly different ephippial morphology; they almost certainly are new, undescribed species of Ceriodaphnia (Berner, unpublished observations).

In many respects, C. reticulata and C. dubia are very similar: except for pectenation of the claw, their postabdomens are quite alike. The only other striking differences are in the morphology of the antennules, especially in the males. Their ephippia are alike in having tiny spinules over the surface, while the bumpy tubercles obvious on the mature reticulata ephippium are not prominent during the period in which the females will copulate. Goulden (1966) has suggested that male cladocerans explore the ephippial surface prior to coplation as a means of mate identification. This has not been observed in Ceriodaphnia, but if it occurs, one can imagine, given the similarity in ephippial surfaces, that these species might mismate. In addition, the morphological similarities between C. reticulata and dubia could be interpreted as evidence that they share a common ancestor in Ceriodaphnia evolution. If so, there would be a greater probability of their producing viable, fertile hybrids as a consequence of interspecific matings.

An hypothesis at the start of this study was that the third Ceriodaphnia in EPA cultures, which has an ovate-toothed pecten, is such a hybrid. Its close similarity to C. dubia, especially in the male characters, and the fact that females can express either the toothed pecten or a finely-setulated pecten argues against that possibility. Discovery of this form in field populations of C. dubia lacking co-existing C. reticulata suggests that it is a naturally occurring phenotypic variant of that species. A conclusion of this study, therefore, is that this form is not a hybrid, but is probably a phenotypic variant of C. dubia Richard 1894, herewith designated as the toothed-pecten variety.

It seems quite likely that the toothed-pecten morph arrived along with C. dubia as a contaminant of the original C. reticulata cultures at the EPA Laboratory-Duluth. From there it was disseminated to the Athens, GA, EPA laboratory, and to ones at Shell Development and Ecological Analysts, Inc. The morphology of the pecten in this variety of C. dubia appears to be under

environmental control: females had ovate-toothed pectens when cultures from the Athens, GA, laboratory arrived at the Newtown Facility. After several weeks in reconstituted water, the animals all had a dubia-like pecten. When the animals were returned to culture in well water at Athens, with a similar diet, the female pectens reverted to the ovate form. It is possible that differences in salts or trace minerals in the culture media effected the change. (Complete analyses of the culture waters involved were not available). As both forms of the pecten occur simultaneously, but to greater and lesser degrees in natural populations (D. Berner, unpublished observations), this phenomenon deserves further study.

It is possible that the toothed-pecten morph occurs rather widely across the continent but is not recognized or identified correctly. Murdoch et al (1984), for instance, reported the use of C. reticulata in field and laboratory experiments in southern California. Recent examination of their samples by this author revealed that they had, instead, populations of C. dubia, many of which were the toothed-pecten morph and easily mistaken for C. reticulata.

As an outcome of this study, the following recommendations are made:

1. There needs to be a nationally organized and funded program for sampling freshwaters, accompanied by support for systematic studies, development of reference collections, and the publication of adequate taxonomic keys. Difficulties encountered by EPA personnel in identifying the species in their cultures reflect the inadequacy of the keys (Brooks 1959 and Pennak 1978) commonly used in N. America to identify zooplankton. Furthermore, original species' descriptions are frequently in Latin, German, or French, are not readily available, and may not be applicable to N. America because they are of taxa from other continents. Reference field populations were obtained for this study largely because individual investigators were kind enough to share their research materials. This author feels strongly that, as the need to monitor freshwaters increases, so does our need for contemporary, comprehensive reference collections and taxonomic information.

2. To determine whether or not C. reticulata and C. dubia can hybridize successfully, more experiments of the type proposed in this study should be done. The techniques described by Ivleva (1969) could be used to obtain more abundantly gamogenetic populations. In addition, ephippia resulting from

interspecific matings should be collected, hatched and reared (Leonhard and Lawrence 1981) to see if viable, reproductive hybrids can be produced. The hybrids morphology should be compared with that of the parent species.

3. It is suggested that the form of pecten in C. dubia is under nutritional control. This could possibly be tested by rearing them in defined medium and feeding them algae also grown in defined medium (Keating and Dagbusan 1984). This would make it possible to manipulate the presence and concentrations of micronutrients, which seem likely candidates for such control. Such studies might contribute to our knowledge of some of the factors affecting polymorphism in the Daphniidae.

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APPENDIX A

Taxonomic Characters of *Ceriodaphnia* Used in This Study

Parthenogenetic female (Illustrated in Figure 1)

From the lateral aspect, the body form is rounded to oval, sometimes slightly flattened dorsally and ventrally. The head is depressed; the frons in front of the compound eye is rounded and occasionally angular anterior to the antennule. The ocellus (simple eye) is small, lying between the eye and antennule. There may be a supraoptical depression on the anterior surface of the head below the origin of the fornix, a lateral extension of the headshield which arches posteriorally above the antenna. Dorsally, a conspicuous cervical notch separates the head from the posterior headshield; on its anterior surface an oval headpore, or "fenestra", is frequently visible. The posterior headshield bulges where it overlies the heart (cardiac bulge) and is separated from the dorsal carapace by a usually distinct ecdysial line, which splits when the animal moults.

The carapace is more or less strongly arched along the dorsal margin where the two shells are fused. Dorsolaterally, it bulges out to accommodate eggs in the brood chamber. Posteriorally, the dorsal margin extends to the dorsoposterior angle, where the shells separate. This angle forms a blunt point or pronounced spine, and may lie well above or close to the horizontal axis of the body, depending upon species and maturity of the individual. The carapace and, to varying extent, the head and headshield are "reticulated" by 5- to 7-sided polygons with raised edges and minutely patterned surfaces. The anterior, ventral, and posterior free margins of the carapace curve into each other without abrupt angles or distinguishing landmarks. Just inside the carapace, close to the margin, runs a row of fine spines and spinules which enlarge to form plumose setae toward the posterior portion of the ventral edge.

The antennule (first appendage) is small and moveable, varying in size and shape with species. It has a terminal cluster of 9 aesthetascs (fine sensory setae) and a longer, anterior sensory seta located slightly proximal to the apex. The antenna (second appendage, used for swimming) has a basal

segment and two rami of 4 and 3 segments. The latter bear long, 2-segmented, plumose setae according to the formula: exopod (dorsal ramus) 0-0-1-3; endopod (ventral ramus) 1-1-3. Hidden under the carapace there are five pairs of branchial appendages for feeding and respiration which were not examined in this study except in the male (see below).

The posterior, dorsal portion of the body bears a single abdominal appendage which helps retain eggs in the brood chamber and may be insignificant in size or long and tapered. Posterior to it, a pair of long, plumose abdominal setae protrude from under the carapace and mark the beginning of the tapered postabdomen, which terminates with a pair of curved claws. Near its midpoint, the dorsal margin has a slight or strong inflection, depending on species. Posterior to this, it splits to form the margins of the anal opening, then curves , sometimes with a flare, ventrally to the claw. The spinulation of the postabdomen is significantly different among species. Denticles arm the anal margins and the posterior curve of the dorsal margin; they are generally longest along the curve and vary in length, weight, and curvature. Just ventral to them, on the lateral surfaces of the postabdomen, are rows and clusters of fine spinules that run proximally toward the dorsal inflection where, depending on species, they become heavier and more scattered. Each claw has a row of fine spinules on its medial and lateral surfaces. The medial row is undifferentiated among species. The lateral row consists of three sections, or pectens, that vary in length, weight, and size among certain species.

Sexual (ephippial) female (Refer to Figures 2.2, 6.2, 10.2)

In the sexual female, the upper half of the carapace is modified to form an ephippium, a saddle-shaped protective case for the single 'resting' embryo resulting from mating. The ephippium develops during a 'sterile' instar in which no parthenogenetic eggs develop in the brood chamber; during this stage the dark, haploid egg can be seen developing in one ovary lateral to the gut and the dorsal margin of the carapace appears somewhat raised and pinched laterally. Development of the ephippium, which has a somewhat triangular shape and a straight dorsal margin, pushes the ecdysial line forward and depresses the head more ventrally than in the parthenogenetic female.

The margin of the ephippium is composed of shallow cells. Above these is

a semicircular collar of deep, polygonal cells enclosing a region of shallower cells that bulges out to form a 'locule', in which the embryo is encased after the carapace is moulted and the ephippium pulls free. Among species, the surfaces of the polygonal cells and the locule differ in decoration by tufts, hairs, tiny spinules and other modifications. In addition, the dorsal ridge where the two sides of the ephippium meet may vary in height and prominence among species. Depending on species, the ephippium of living specimens frequently has an orange-greyish color.

The antennule and postabdomen are as in the parthenogenetic female.

Male (Refer to Figures 2.3, 6.3, 10.3)

Males strongly resemble immature females in size and shape: from lateral aspect they are almost quadrangular in shape, with a flat dorsal margin between cervical notch and posterodorsal angle. The latter is extended into a distinct point which sometimes bears small spines. The head and compound eye are larger than in juvenile females, and the posterior portion of the body is more compressed laterally. The fornix, antenna, and postabdomen are usually like those of the female.

Aside from the head and large eye, males are distinguished by modifications of the antennule and first thoracic appendage. The antennule is longer than in the female and bears at its apex a special, 2-segmented 'male' seta. The aesthetascs lie slightly proximal to this on the posterior surface of the antenna and the longer, sensory seta is proximal to these on the anterior surface. The size and shape of the male antennule and its distal seta varies among species and may be quite distinctive in some. The first thoracic appendage bears a small, inconspicuous hook, hard to see in undissected specimens, and a long, thin, 2-segmented clasper. This reaches nearly to the posteroventral curve of the carapace when folded inside the shell and is very conspicuous when extended outside the animal.

In culture, males are often recognizable by their rapid, erratic swimming habits, their denser coloration than the females, and their extended antennules and claspers.

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