Tardigrades: In the Classroom, Laboratory, and on the Internet

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Abstract: Developing students' understanding of the nature of science is an objective of all high quality science instruction. Recent efforts to reform science education in the United States have strongly emphasized this understanding as an essential component of general scientific literacy. The Kansas Collaborative Research Network (KanCRN) is an open, internet-based, community of researchers, teachers, and students who believe that the best way to understand the nature of science is to engage in ongoing research. During the development of the Tardigrade Survey section we were faced with questions of how to present projects and challenges to high school level students that would stimulate their interest in and foster their investigatory approach to science without being to prescriptive or didactic. Our desire was to help students become active researchers, collaborating with others and developing and using skills of scientific inquiry to conduct research. Several key components are used in the project: collaborative research coordinated through the website, interaction with a fully qualified Mentor, a of set standardized protocols and references, a key to the animals, a data review process, an accessible database, a comprehensive plan for curation of the specimens, and a multi-leveled plan of recognition, reporting, and publication. We have designed a model for collaborative research directed to achieving these goals and by use of the Internet will be available and useable by schools around the world.

Keywords: tardigrades, KanCRN, collaboration, Internet

Introduction

As one surfs the World Wide Web for almost any subject, the result is so many matches that the enormity of information is unfathomable. Even on such an obscure and little known phylum of animals as the *Tardigrada* (Figure 1) we got several hundred "hits" most of which were of questionable value. We also found the photographs our colleagues displayed without acknowledgment or permission. Since the content of most scholarly journals and books is not yet available on the net it was hard to envision anything of value coming from this environment, but sprinkled amongst the time wasters we found some glimmers of hope.

Recent efforts to reform science education in the United States have strongly emphasized understanding as an essential component of general scientific literacy (AAAS, 1993; NRC, 1996). The Kansas Collaborative Research Network, (KanCRN) (pronounced like: kancern) http://kancrn.org, is an open, Internet-based, community of researchers, teachers, and students who believe that the best way to understand the nature of science is to engage in ongoing research.

The KanCRN project is an example demonstrating good intentions but with scientific weakness. They developed a good project that involves measuring the lichen coverage on trees and interpreting the degree of coverage as a bioindicator of atmospheric sulfur dioxide. The project then recommends counting and identifying the "types" of tardigrades living in the lichen in order to prepare diversity and density statements. Then the results were to be posted to a database on the net. Because "type" is not a recognized taxonomic classification, the identifications would not be consistent between sites and calculations that followed would be uncomparable. The identification data from the tardigrades had no review for validation. In this process, hundreds of high schools doing this project and posting their data to the net in such an uncontrolled fashion would only add to glut of confusing information on the World Wide Web. The data would be of little value to science and the students would be learning to do "bad" science, without realizing the long range consequences.

To give scientific value to their project, collaboration was developed between KanCRN and the authors to address the issues and develop a site and projects that have the rigorous requirements of "good" science incorporating appropriate levels of review and obtaining data which will be acceptable to the international scientific community. First, we modeled the way most classical science research is conducted in universities and secondary schools (Figure 2A). The model shows little or no communication between secondary schools, colleges, and universities except where the secondary teacher or student finds references in a library. However, the Internet has changed the dynamic and today's secondary students not only can but also enjoy "surfing the net" for reference information. What they find is often plagiarized or out-of-context snips of information posted by non-experts and as such of little real value. With KanCRN, we developed a research model for collaboration (Figure 2B) which uses the tools of the internet, is supportable by scientific researchers, and delivers real world projects to secondary schools. Our dream is to have designed a model that other groups may build on to bring science to the classroom and valid scientific results to the world. This report is the result of that work.

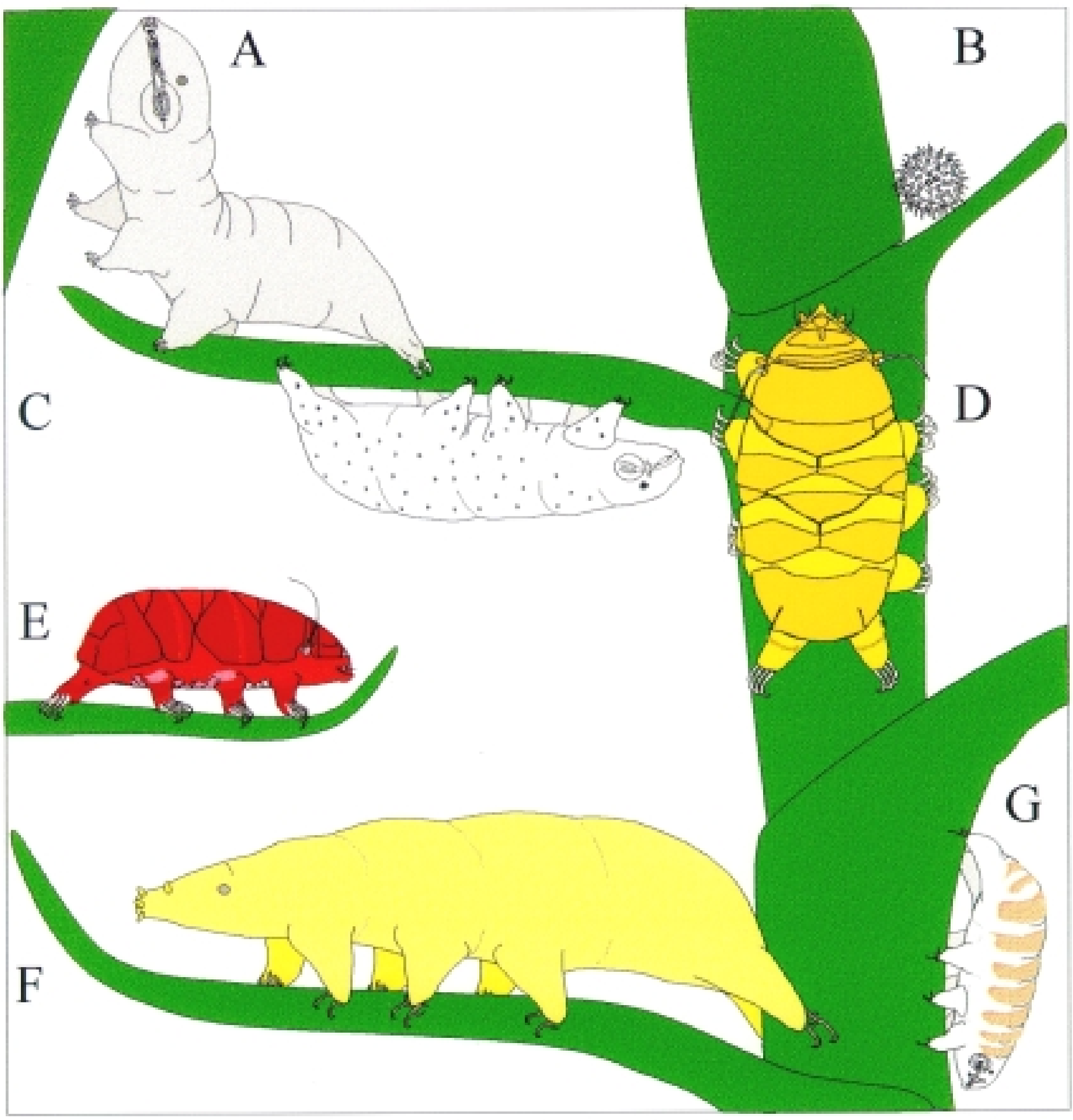

Figure 1. *Drawing of tardigrades in natural habitat between the leaves of a moss plant. A. Macrobiotus, B. tardigrade egg, C. Minibiotus, D. Echiniscus, E. Pseudechiniscus, F. Milnesium, G. Ramazzottius*

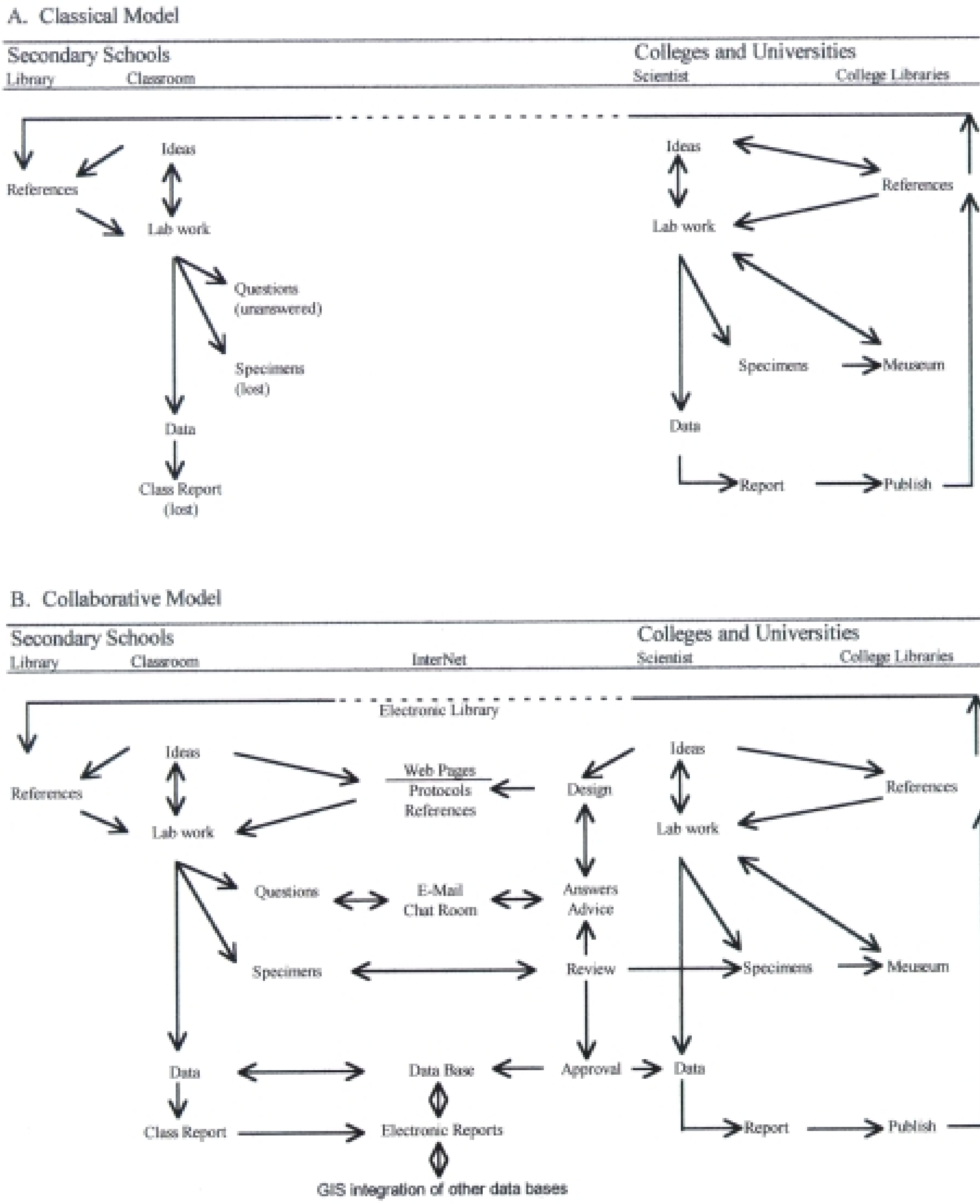

Figure 2. *Research Models. A. Classical Model demonstrating little communication between college and university research communities and secondary schools. B. Collaborative Model demonstrating how the Internet provides both a means and value to both parties for collaborating on research.*

The Tardigrade

The tardigrade or waterbear (Figure 3A, 3B) is a microscopic, aquatic animal that is found living in the interstitial water that becomes trapped between the leaves of moss plants and the thalli of lichens. In this micro-environment is a whole community of other animals, including protozoans, rotifers, nematodes, mites, collembola, and insect larva. Here the active state of the tardigrade lives, moves, eats, grows, reproduces, and die. Here they survive the changes of seasons by drying and moistening with the environment. They use cryptobiosus to get through these dry times by withdrawing into their cuticle and shriveling into a stage called a "tun". In this stage they have been experimentally subjected to extreme conditions of heat (120 C), cold (-200 C), pressure (20,000 psi), and excessive concentrations of common gases and been revived to the active stage (Kinchin, 1994).

Despite its small size (200-700 micrometers) the tardigrade is easily recognized by its body shape and four pair of legs each ending in claws (Figure 3C). They look like minute caterpillars or little bears hence their name "waterbear". Tardigrade means "slow walker" which accurately describes the deliberate and lumbering nature of their movement. In a world of fast moving, wildly wiggling, and undulating critters, the tardigrade represents a vision of bigger more "standard" animals. It has legs and moves by walking and it crawls over, under and around debris (Miller, 1997). It eats both plants and animals, has separate sexes and lays beautiful ornate eggs with great conical spines and projections that look like little, sparkling Christmas tree ornaments when seen under the dissecting scope (Figure 3D).

Tardigrades are easy to work with because they are so forgiving. They can be collected by scraping a lichen from a rock or tree with a pocket knife or by plucking a clump of moss from its substrate and placing the sample in a paper bag or envelope to dry. The animal will slip into cryptobiosis if it is active and can be revived in the laboratory by placing it in a dish or cup of distilled water. Active tardigrades may generally be found in an hour or two by scanning the debris and dish bottom with a 40 power dissecting scope. A water mount slide may be easily made by placing a drop of water containing the tardigrade on a slide and adding a coverslip. For large animals, it may

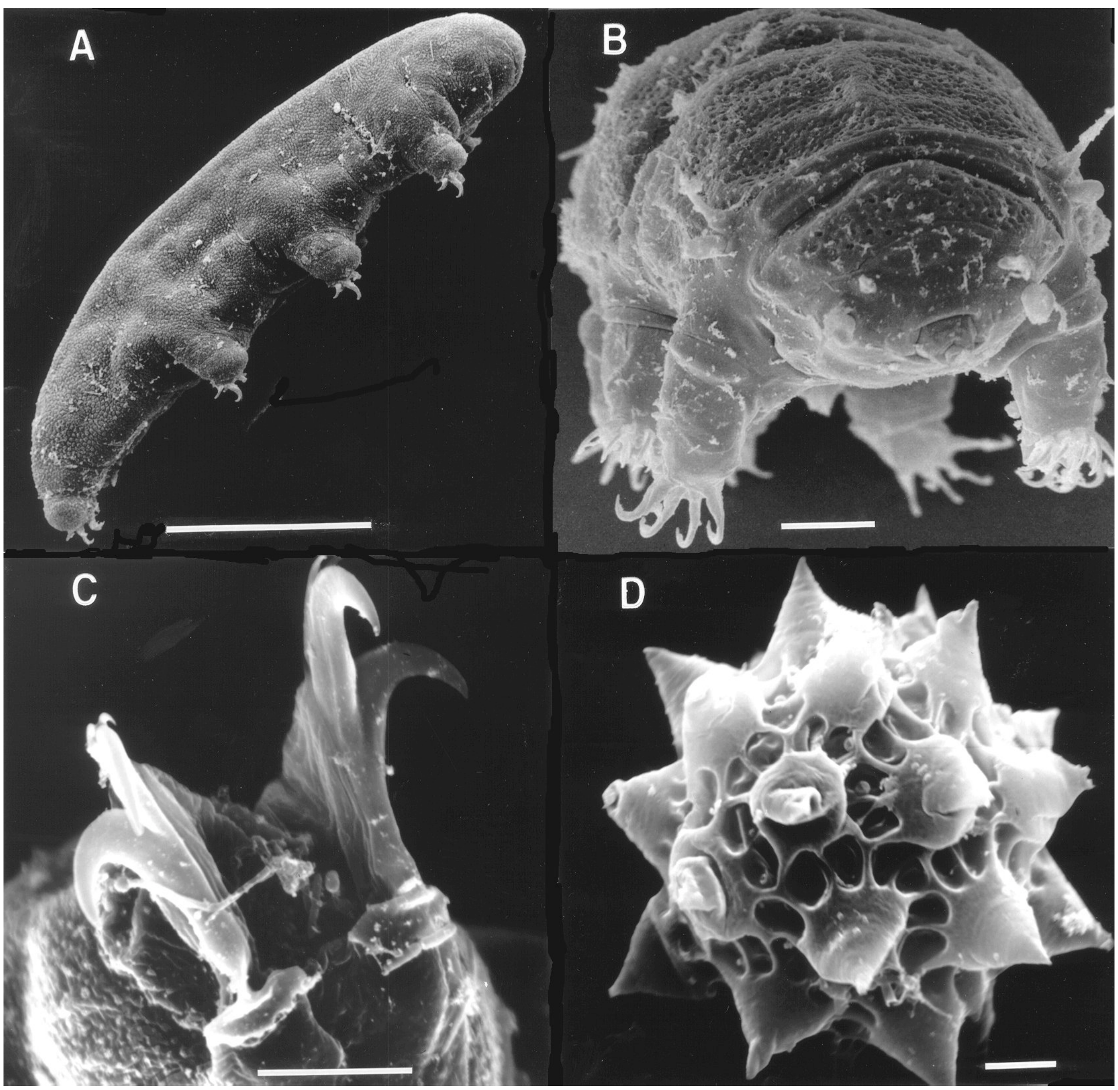

***Figure 3**. Scanning Elecrton Microscope (SEM) photomicrographs of tardigrades. A. Side body view of an adult Eutardigrade, scale bar = 75 µm; B. head view of a Heterotardigrade, scale bar = 10 µm; C. claws of a Eutardigrade, scale bar = 10 µm; D. Egg of a Eutardigrade, scale bar = 10 µm.*

be necessary to place a couple of coverslip fragments on the slide to support the main coverslip and provide a space for the animal to move about. This is great way to observe the workings of their internal organs, muscles, feeding apparatus, and movement. If a video camera attachment is available for the microscope it enables many students to observe and discuss the material (Pennak, 1978).

After 24 to 48 hours, most specimens will asphyxiate, a stage of life where they lose osmotic control and absorb too much water, become turgid, and bloat like a spaceman with a suit full of air. This is a survival stage to get through times of too much water. In this stage they are easily transferred to 70% alcohol or 4% formalin for preservation or to a microscope slide in a mounting media. The slide should be allowed to dry for a week or two then the coverslip sealed with epoxy paint to prevent further drying of the media (Miller, 1997).

Tardigrades may be identified to family and generally genus with slide mounted specimens and a student level microscope at 400 power. Both the external and internal structures and features are readily visible in most specimens although because of orientation it may be necessary to look at several specimens from the same sample to answer all questions in a key. Identification to species is somewhat more challenging, some groups require the eggs to differentiate species while others may need to be observed with research level optics of phase contrast or differential contrast microscopes (Figure 4A, B, C, D). The most recent monograph was published in Italian (Ramazzotti & Maucci, 1982) but there is an English translation is available. There have been a considerable number of new species describe and taxonomic rearrangements effected since then.

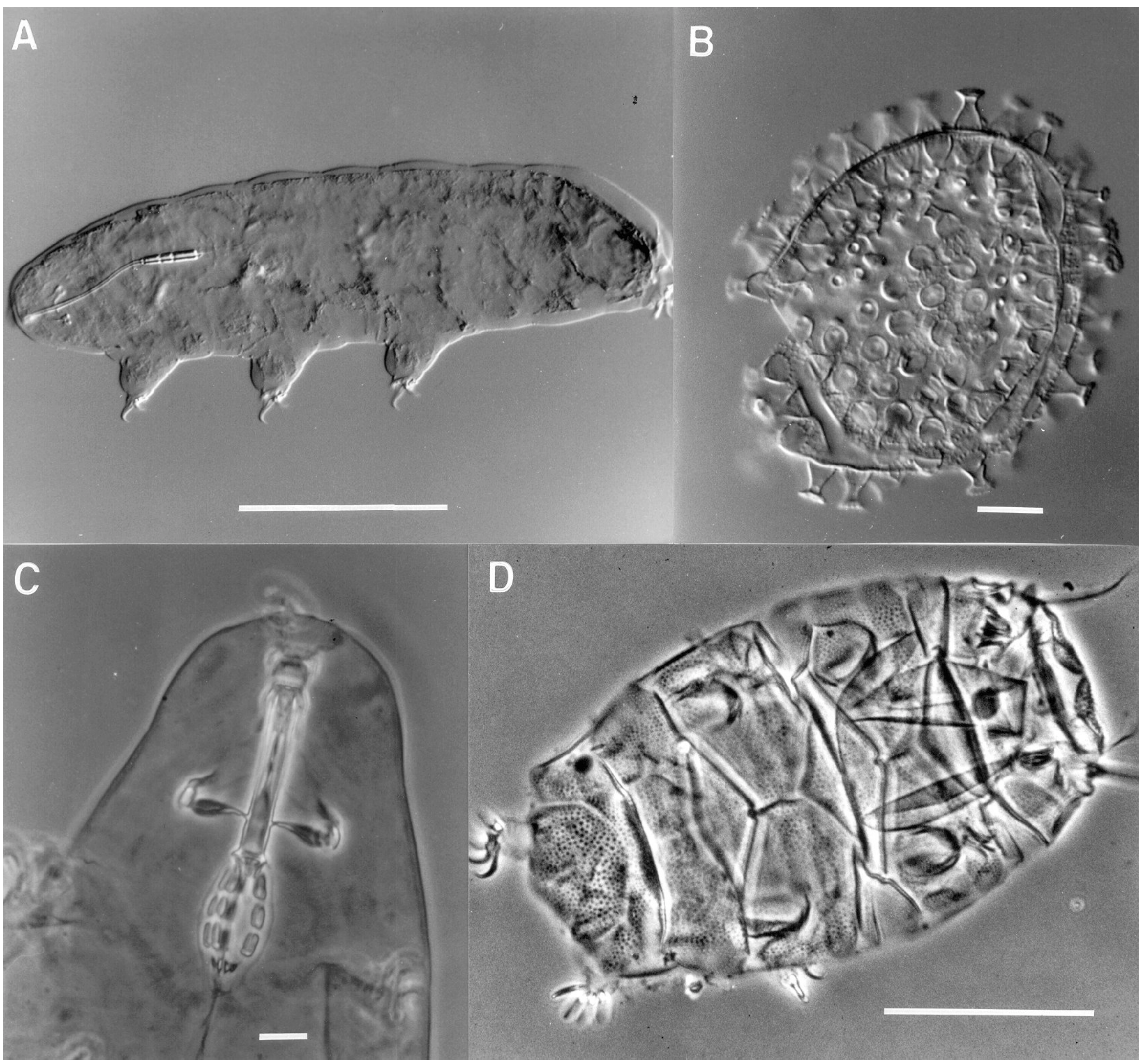

Figure 4. *Light Microscope photomicrographs of tardigrades. A. Side Body view of an adult Eutardigrade, scale bar = 100 µm; B. egg of a Eutardigrade, scale bar = 10 µm; C. mouth parts of a Eutardigrade, scale bar = 10 µm; D. dorsal plates of a Heterotardigrade, scale bar = 100 µm.*

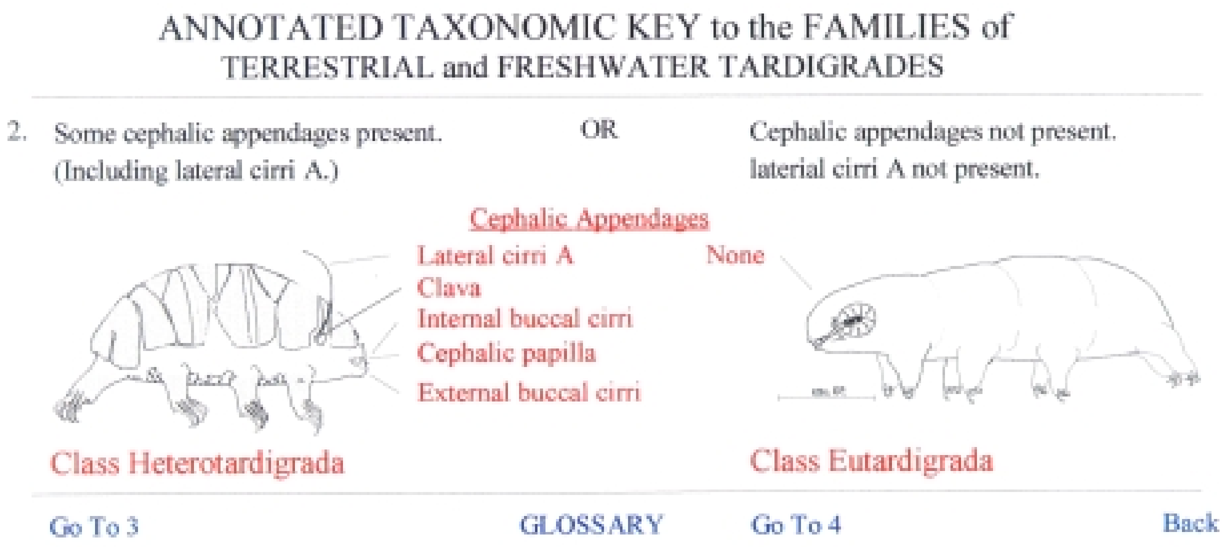

Figure 5. *Panel from the Annotated Taxonomic Key to the Families of Terrestrial and Freshwater Tardigrades available online at the KanCRN site.*

The Project

In order to achieve our goal of doing "good" science to improve student learning, we first had to define the scope of the project. We decided to build on the previous SO_2 project and limit the initial work to lichens, and the tardigrades found in them. This has two advantages, first the lichen is a relatively clean environment and when soaked does not generally produce the cloudy hard to see through sample that moss associated with soil does. And second, the project is not initially confused with the other common tardigrade habitats of moss, leaf litter, soil, sand, fresh water, and salt water.

Next we built and placed online an annotated dichotomous key to the families of tardigrades to bring the "type" question, into taxonomic terms (Figure 5). The key and its supportive descriptions with pictures and drawings will be expanded based on student collected specimens. That is, a new species and its description will be added to the key and database. The students will participate in the building of those resources, and get credit for their work. State species lists will be maintained and the "discoverers" will be recorded.

To provide basic background information we obtained permission from the *Kansas School Naturalist* to post the entire contents of their May 1997 issue, "Tardigrades: Bears of the Moss" (Miller, 1997), on our site to be read or printed out. Next we developed teacher support, to both learn and teach about tardigrades, by posting a Power Point slide show of 30 slides that explains tardigrade morphology, structure, classification, capability, stages of life, and provides references (Miller, 1998). The slide show can be run from the site or it can be downloaded and made into overheads. We have provided the note cards with the downloaded version and it is in both color and black and white. We have obtained permission to post online, a five minute video made at James Cooke University, Townsville, Australia, on how to handle and process tardigrades (Miller et al., 1993). Finally, the many terms used in the key also required that we build a glossary such that descriptions that are more detailed and explanations could be provided.

Recognizing that there will be a variety of ages and skill levels in the collaborators we designed a set of protocols that can be used as a progressive series or discrete units. The first level consists of simply contributing lichen samples and getting a report bac. Second is a general Tardigrade lab; a training exercise to collect, process, find and view the animal. This enables the teacher and students to determine whether they have the equipment and background to tackle projects that are more advanced and desire for further involvement. The third level of involvement is to process and identify the tardigrades found. The fourth level is advanced projects for classes or students who have built some experience through other levels and are ready to pose and test their own hypothesis using tardigrades and research tools that have been provided. These projects will be worked out with the Project Mentor and are suitable for science fairs and other presentations.

The Project Mentor is a specialist in the area who will communicate with each class or student by E-Mail and chat room to help with questions and challenges. This student scientist partnership will help insure the quality of the work. The Project Mentor will receive the samples and specimens, verify them, and return a report to the class or student. The report will include Museum quality Reference slides of the animals found; drawings, descriptions, and pictures of the animals found; and recognition for the contribution to the survey. The Mentor will select representative specimens to go to the permanent collection.

When students have completed the requirements of the selected protocol, they will have one or more data sheets. These will be entered into the Data Submission Webpage of KanCRN Tardigrade Survey Database. The samples or specimens will be sent to the Mentor for review and verification. The meeting point of each project is the Mentor. Because for the work of a class or student to be moved from submitted to accepted in the KanCRN Tardigrade Database, it must be reviewed and approved by the Mentor. This is as simple as sending part of the collected lichen sample and as complicated as sending identified slide specimens to be verified. The members of the world tardigrade community are aware of the project because it was outlined in their newsletter in June of 1998 (Slow Walker News, 1998). By survey response, most researchers have indicated that they do not want to be inundated with direct requests for advice, literature, and identifications but are very willing to provide identification of specimens and other support through the Mentor.

The University of Kansas Natural History Museum, Division of Invertebrate Zoology has agreed to accept, house, and curate the permanent collection of slides contributed by the KanCRN Tardigrade Survey project. This solves another question of science, the long term availability of work for later review. Secondary schools and even most colleges do not have the resources of space, time, and knowledge to maintain a collection, so we are very grateful to the University of Kansas Natural History Museum for volunteering to support this project and their commitment to build a world class tardigrade reference collection. Here the specimens will be cared for and made available to researchers and collaborators.

Data will be available from the Tardigrade Database. Students will be able to access the entire data set but data that has not been reviewed by the Mentor would be tagged as "unconfirmed". Students would then begin analysis by comparing confirmed data with unconfirmed sets. The database of results will allow the tardigrade data to be opened and compared graphically using a Geographic Information System, GIS, format. GIS integrates common database operations such as query and statistical analysis with the unique visualization and geographic analysis benefits offered by maps. Mapping can occur online through the KanCRN website or with the Arc-View GIS software being made available to the schools through the program. With this access and using only confirmed data, students can ask and answer additional questions that emerge from mapping tardigrade family, genus, and eventually species distribution, density, and diversity data vs. weather, geology, geography, vegetation, pollution, population, and roads.

The last area of concern is reporting and publication. The class or student will file an end of year report to KanCRN on what has been learned and discovered; those reports will also be submitted electronically. Selected students will be asked by the Mentor to present their work as posters and papers at the annual KanCRN research meeting and state academies of science. Some may be asked to co-author a scientific paper to be submitted to a reviewed journal. KanCRN has committed to select and send a teacher and student who best represent the project to present a complete report to the world tardigrade community at their next international meeting in Copenhagen, Denmark, in the summer of 2000.

Student scientist partnerships like the tardigrade project are one example of how biology classrooms are making a transition from learning about science, to doing science. Dr. Neal Lane (1997), as Director of the National Science Foundation, said it is clear to the science community that they must "look beyond the traditions of research in higher education and to understand and explore the possibilities of new partnerships between research and education at the primary and secondary levels." The communication technology of the World Wide Web is also allowing the development of a powerful tool to help students to create and participate in a research community.

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