

NORTH AMERICAN BENTHOLOGICAL SOCIETY

PLECOPTERA TECHNICAL WORKSHOP

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SCHEDULE AND TOPICS

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INTRODUCTION AND HISTORY

R. Edward DeWalt

The order name for stoneflies, Plecoptera, comes from the Latin *plecto*, meaning plaited, braided, or folded and the Greek *pteron*, meaning feather or wing, and refers to the ability of adults to fold wings on their back. Compare this to mayflies (hold wings vertically) and dragonflies (wings held horizontal), where wings do not fold. Folding the wings is an apomorphy of several orders collectively called the Neoptera.

The order Plecoptera is defined by several internal structures (discussed in the Phylogeny chapter) that most non-specialist will never observe. General characteristics that will help non-specialists recognize an insect as a stonefly include a combination of two stout cercae (a.k.a. tails, the medial filament of mayflies is absent, but, some stonefly families (outside NA) have a finger-like gill on the abdomen tip); tarsi three-segmented; tarsal claws two; and gills, if present, are finger-like (not plate-like as in mayflies), possibly clustered and located on the submentum (ventral head), cervical membrane (membrane between head and thorax), thorax, or abdomen. Adults have the same body plan as do nymphs, but most have wings of various lengths (males of *Allocaonia vivipara* (Claassen) and some other species are wingless). Adults have the shriveled remnants of nymphal gills, a characteristic that is of great use in their identification.

Stoneflies are an ancient order. Their fossil record extends back into the Permian, about 250 million years ago. Baltic amber from the Miocene (38-54 million years ago) has produced specimens of several extant families (Zwick 2000). While the area of origin of Plecoptera is unknown, the suborder names Antarctoperlaria and Arctoperlaria suggest that the distributions of the suborders surround the Antarctic and the Arctic, respectively. Arctoperlaria occur throughout the northern hemisphere and Oriental region with two families reaching into the southern hemisphere. Antarctoperlaria is generally distributed across the southern hemisphere, but does not currently have representatives in the northern hemisphere. The breakup of the landmass Pangea has had a major hand in how the suborders were distributed. Extinction too, has enforced limits on current distributions, the families that have made it to modern times, and our ability to reconstruct stonefly phylogeny.

Stoneflies have been the object of scientific study for a very long time, with the first species being described by Linnaeus in 1758 and placed within the genus *Phryganea* of the Trichoptera. Burmeister first proposed the order name Plecoptera in 1839. The order now has over 2,000 described species (Zwick 2000). Because of this early start and many earnest stonefly researchers over the past 350 years, stoneflies are one of the best known orders of insects. This is supported by the three world catalogues produced in the 20th Century summarizing nomenclature and providing a synthesis of the order. In North America, there are approximately 630 known species, with several being described yearly. However, the greatest numbers of new species are now coming from Asia, especially China. Much work remains to be done in this area and there are several capable Asian plecopterologists at work presently.

The first description of a North American species was by Linnaeus in 1758 of *Diura bicaudata* (as *Phryganea*), a species of Holarctic (combined Nearctic and Palearctic realms) distribution. Say, in 1823, described four species from four families. It was 15 years later that Newman's descriptions of six more species were added. Pictet added several more species in 1841, and this is how it went for several more decades—increasing species and knowledge of stoneflies at a snail's pace. Nathan Banks was the first major stonefly researcher in North

America. He began publishing in 1895, continuing until 1948. His efforts dramatically increased the known stonefly fauna by 66 species, having contributed to all nine families with a preponderance in the Perlidae (11 spp.) and Perlodidae (17 spp.). His efforts jumpstarted the study of stonefly systematics that has seen only a few lulls since his time (Fig. 1).

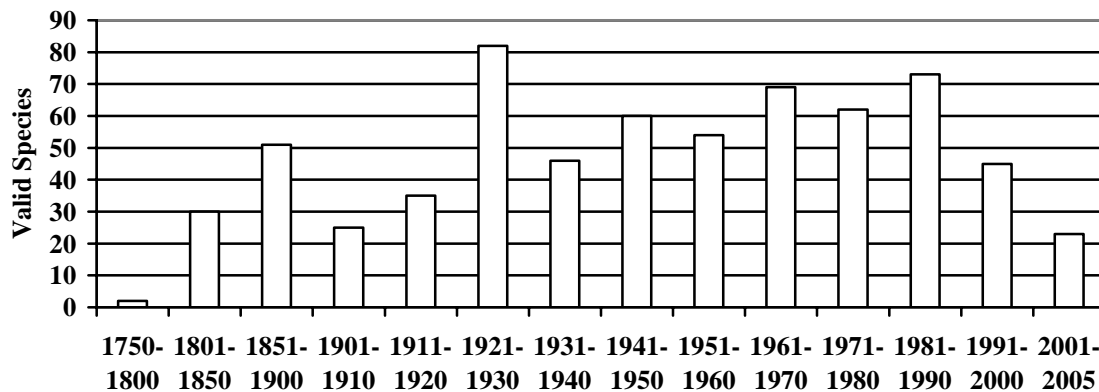


Fig. 1. Number of valid species added in each time frame. Note that the first time frames are of different intervals than than succeeding ones and the last time frame is incomplete.

James G. Needham began his work on stoneflies about 1900 at Cornell University. He and his student Peter W. Claassen published an important monograph on the stoneflies of America north of Mexico in (Needham & Claassen 1925) after several years of adding new species. Claassen, who died unexpectedly, had his world catalogue published posthumously (Claassen 1940).

Theodore H. Frison, of the Illinois Natural History Survey, in the period 1924-1944, described 58 valid species, built a world class collection, and published on Illinois winter stoneflies (Frison 1929) and a statewide identification manual (Frison 1935). The latter had copious notes on life history and ecology. William E. Ricker, who died only recently, was a force in stonefly systematics and biogeography in North America between the late 1930s and 1980. He is the sole author or coauthor of 86 valid species and is responsible for the creation of many subgenera that were later elevated to generic status. He and Herbert Ross revised several genera of winter stoneflies (*Taeniopteryx*, *Zealeuctra*, *Allocapnia*) in North America. A culmination of this work was a systematic and biogeographic treatment of the genus *Allocapnia*, with hypotheses for post-glacial dispersion of the genus (Ross & Ricker 1971). Illies (1966) published a second world catalogue, the result of which for North American fauna was the elevation to generic status of many of Needham and Claassen's (1925) and Ricker's (1952) subgenera. Others who made major contributions in North America were Jewett (Northwestern NA stonefly distributions, descriptions, and biogeography), Gaufin (Utah and Rocky Mountains stoneflies), and Hitchcock (stoneflies of Connecticut).

Another world catalogue and phylogenetic system was published Zwick (1973). This established the present suborder relationship (Antarctoperlaria and Arctoperlaria) and set the major groupings of the Arctoperlaria (Euholognatha and Systellognatha, what we have in North America). At this time, Richard (Dick) Baumann, Brigham Young University, began his life's work on the Nemouridae and western mountain stoneflies (Baumann et al. 1977). In the late 1960s, Kenneth Stewart, University of North Texas, began working on the ecology, behavior, and nymphal descriptions of North American species. Bill Stark, Mississippi College, began his

career in the 1970s, tackling the tough revisionary work on genera of perlids, peltoperlids, and perlodids. He is truly a student of the world fauna with his work on *Anacroneuria* Central and South America and in Asia. He, with Stewart (Stewart and Stark 2002) have dramatically improved the knowledge of North American stonefly nymphs. Stan Szczytko, University of Wisconsin at Steven Point, began his life's work on the *Isoperla* and closely related taxa in the 1970s. We all hope to hear that an eastern companion to his western North American *Isoperla* monograph (Szczytko and Stewart 1979) is going to press soon. Peter Harper, of the University of Montreal, has contributed greatly to the knowledge of the stoneflies of eastern Canada through his descriptions of nymphs and life history studies.

Boris Kondratieff, often with Fred Kirchner, has published 27 species descriptions between 1979-2004, published regional faunal studies, and completed revisions of several genera. Charlie Nelson has contributed revisions to the Pteronarcyidae and *Hydroperla* and provided the first cladistic analysis of North American stoneflies. Riley Nelson has completed several fine revisions to western North American Capniidae in the 1980 and 1990s. Other workers have contributed significantly, but the scope of this manual is such that not all of them can be acknowledged here. You will meet some of the newer workers at this workshop.

Given that this is a one day workshop, this manual and presentations are intended to provide broad strokes on stonefly biology. We have included a references section with key literature on stoneflies that those who wish to know more should consult.

PLECOPTERA PHYLOGENY AND CLASSIFICATION

Kevin Alexander

The more than 2,000 species of Plecoptera constitute a monophyletic group within the neopterous (wings fold flat over back) insects and have recently been reviewed by Zwick (2000). This overview primarily follows Zwick (2000) but much of the phylogeny of Plecoptera is under debate and alternative phylogenies have been proposed (Nelson 2005, Thomas et al. 2000, Uchida and Isobe 1989; Zwick 1973)

The exact relationship of Plecoptera to other insect orders has been greatly debated but no definitive resolution has been reached. Moreover, in attempts to support the monophyly of Plecoptera, the identification of unique derived characters that could be used to justify this clade has been difficult. Plecoptera have typically been defined by sets of ancestral characters. Zwick (2000) does defend the monophyly of Plecoptera with a several primarily internal or subtle morphological characters. These include: gonads forming a loop in both males and females; the musculature in nymphs that allows laterally undulating swimming; the loss of an ovipositor; and, a circulatory organ at the tip of the abdomen.

Zwick (1973) has recognized 16 families within two monophyletic suborders, the Antartoperlaria and the Arctoperlaria. The Antartoperlaria is primarily southern hemisphere in distribution as the name implies and contains four families. The Arctoperlaria is primarily northern hemisphere with the exception of the Notonemouridae, which is strictly southern hemisphere, and two notable perlid genera *Anacroneuria* and *Neoperla*, that have substantially expanded their ranges into the southern hemisphere.

The Arctoperlaria is diverse and includes the remaining 12 families of Plecoptera and the vast majority of species. This diversity makes monophyletic characterization difficult, but Zwick (2000) and others have proposed that the evolution of vibrational based mate finding behaviors as the apomorphy that supports monophyly of the Arctoperlaria. This drumming or tremulation produce substrate-born communication between males and females that allows mate recognition and location. These behaviors and associated morphological structures are ubiquitous amongst the Arctoperlaria but are not known from any taxa within the Antartoperlaria, despite extensive studies.

The Arctoperlaria, the focus of this workshop, consists of two main clades: the Euholognatha and the Systellognatha. The Euholognatha encompassing six families, four of which are North American, is defined by an unfused corpus allatum fused to the aorta, a soft egg chorion, and positioning of nerves under longitudinal muscles (Zwick 2000). Most euholognathans are herbivore-detritivores and darkly or drably colored. Within the Nearctic Euholognatha, the Taeniopterygidae is most basal and is characterized by an elongate second tarsal segment, enlargement of the first cercal segment in males, and a small, exposed genital opening of females. The families Nemouridae, Capniidae and Leuctridae have been proposed as the sister clade to the Taeniopterygidae with the Capniidae and Leuctridae being the sister clade to the Nemouridae. Capniids are distinguished by a reduction in crossveins in the wings and the unique shape of the paraprocts, which form a fusion plate. Apomorphic characters in leuctrids include wings rolled around the body, a reduction of cerci to one segment (adults), internal reproductive structures, and the structure of the paraprocts. Nemourids apomorphies include a widened head, labial palps with a disc shaped end segment, transversely enlarged procoxae, and some internal nerve and reproductive structures.

The Nearctic families of the Systellognatha are comprised of the Pteronarcyidae, Peltoperlidae, Perlidae, Perlodidae and Chloroperlidae. This clade is uniquely defined by reduction in adult mouthparts and the male epiproct being sunk into an area that divides tergum ten into hemitergites. Zwick (2000) groups Pteronarcyidae and Peltoperlidae into a clade termed the Pteronarcyioidea that is the sister clade for the yet unresolved trichotomy constituting the Perloidea: families Perlidae, Perlodidae and Chloroperlidae. The Pteronarcyioidea are darkly pigmented herbivore-detritivores, whereas the Perloidea are carnivorous, at least as later instar nymphs, and tend to be boldly or brightly patterned.

The family Pteronarcyidae contains large stoneflies that are distinguished by the reduced sclerotization of the thoracic ventropleurites and by a laterally expanded arolium. Zwick also proposes that the reduction in drumming structures is apomorphic at this level. The peltoperlids have a variety of apomorphies related to their distinctive “cockroach” shape.

The family Perlidae is characterized by the following apomorphies: fusion of both ends of the ventral nerve cord, nymphal head with expanded genae, complex folding of the proventriculus, gill bases with shields, and the type of movement of some of the thoracic muscles. Perlodid apomorphies include the presence of submental gills and the lateral stylets of the penis. Apomorphic for Chloroperlidae is the long slender body, shortened cerci, and an oval pronotum.

Thomas *et al.* (2000), using sequence data from the 18S gene, produced a cladogram to map out the origin of insect flight with stoneflies as a model. The cladogram is substantially different than the phylogeny discussed above. The main differences include no recognition of the subordinal division of Arctoperlaria and Antarctoperlaria and having the family Nemouridae being the sister clade to the rest of Plecoptera with the Taeniopterygidae being the next outgroup within the remaining Plecoptera. Matthew Terry, formerly of Brigham Young University but now at the University of Arizona, has recently produced a molecular based phylogeny of Plecoptera but the results have yet to be published. This study, along with others in the future, may help resolve some of the basic questions about evolutionary relationships within the stoneflies.

CLASSIFICATION OF WORLD PLECOPTERA

<u>Suborder</u>	<u>Antarctoperlaria/Southern</u>	<u>Suborder</u>	<u>Arctoperlaria</u>
Family	Austroperlidae	Group	Euholognatha
	Diamphipnoidae	Family	Capniidae
	Eustheniidae		Leuctridae
	Gripopterygidae		Nemouridae
			Notonemouridae
			Taeniopterygidae
			Scopuridae
		Group	Systellognatha
			Pteronarcyidae
			Peltoperlidae
			Chloroperlidae
			Perlidae
			Perlodidae
			Styloperlidae

WORLD BIOGEOGRAPHY AND DIVERSITY

Dennis Heimdal

Stoneflies are found on every continent in every biogeographical realm except Antarctica (see Fig. 2 for location of realms). The suborders, *Antarctoperlaria* and *Arctoperlaria*, refer to stonefly distributions surrounding Antarctica and the Arctic, respectively (Zwick 2000). The *Antarctoperlaria* is comprised of four families: *Austroperlidae*, *Diamphipnoidae*, *Eustheniidae*, and *Gripopterygidae*, all of which are in the southern hemisphere. The *Arctoperlaria* contains 12 families: *Capniidae*, *Chloroperlidae*, *Leuctridae*, *Nemouridae*, *Notonemouridae*, *Peltoperlidae*, *Perlidae*, *Perlodidae*, *Pteronarcyidae*, *Scopuridae*, *Styloperlidae*, and *Taeniopterygidae*. All *Arctoperlaria* occur in the northern hemisphere with two exceptions, *Notonemouridae*, which is found only in the southern hemisphere, and *Perlidae*, which is found in both hemispheres.

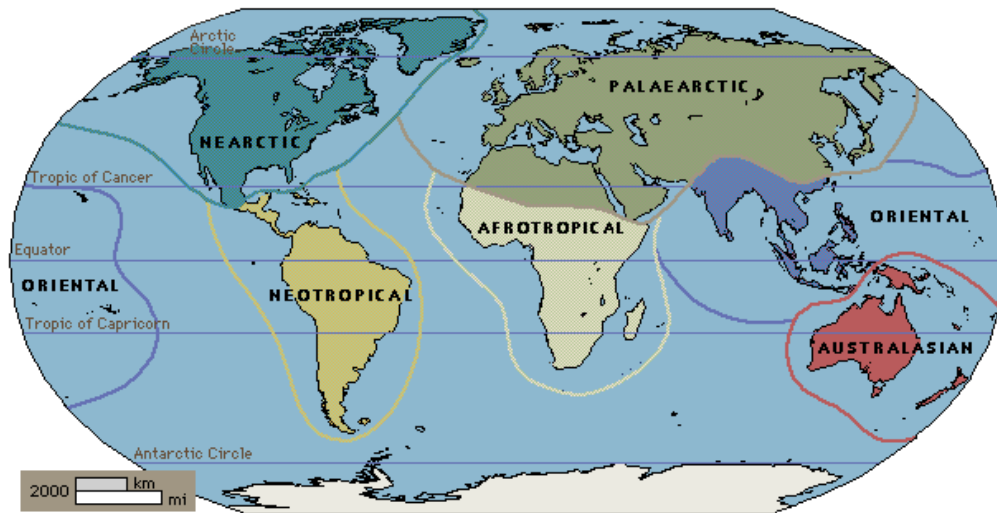


Figure 2. Geographical realms of the world.

The North American Plecoptera

North America is located within the Nearctic realm of the world. There are nine families, 102 genera, and nearly 640 species (Table 1) that occur within North America (Stark 2001b, Stark and Armitage 2004). The greatest diversity within Nearctic stoneflies is found in the Appalachian and Sierra-Cascade Mountain ranges of the United States, in which nearly 50 percent of the endemic genera of North America can be found (Stewart and Stark 2002).

Table 1. Summary of North American Plecoptera.

Family	Genera	Species	Most diverse genera (number of species)
Capniidae	10	152	<i>Capnia</i> (53), <i>Allocapnia</i> (43)
Chloroperlidae	12	89	<i>Alloperla</i> (29), <i>Sweltsa</i> (29)
Leuctridae	9	55	<i>Leuctra</i> (26), <i>Zealeuctra</i> (8)
Nemouridae	12	71	<i>Amphinemura</i> (16), <i>Malenka</i> (11), <i>Zapada</i> (10)
Peltoperlidae	6	20	<i>Soliperla</i> (6), <i>Tallaperla</i> (6), <i>Yoraperla</i> (4)
Perlidae	15	82	<i>Perlesta</i> (22), <i>Acroneuria</i> (18), <i>Neoperla</i> (15)
Perlodidae	30	122	<i>Isoperla</i> (57), <i>Isogenoides</i> (9), <i>Cultus</i> (6)
Pteronarcyidae	2	10	<i>Pteronarcys</i> (8), <i>Pteronarcella</i> (2)
Taeniopterygidae	6	34	<i>Taenionema</i> (12), <i>Taeniopteryx</i> (11)
Total	102	635	

The European Plecoptera

Europe is part of the western Palearctic biogeographic realm. A total of seven families, 34 genera, and about 430 species (Table 2) are present within Europe (Fochetti 2004). The greatest species diversity occurs within Leuctridae and Nemouridae, with nearly 62 percent of the total European species found in those families (Fochetti 2004). The species of *Nemoura*, *Protonemura*, and *Leuctra* are most prevalent within the southern countries of Europe (Fochetti 2004).

Table 2. Summary of European Plecoptera.

Family	Number of genera	Number of species	Most diverse genera within family (number of species)
Capniidae	4	20	<i>Capnioneura</i> (11), <i>Capnia</i> (7)
Chloroperlidae	4	19	<i>Chloroperla</i> (10), <i>Siphonoperla</i> (7)
Leuctridae	3	133	<i>Leuctra</i> (127), <i>Pachyleuctra</i> (3), <i>Tyrrhenoleuctra</i> (3)
Nemouridae	3	132	<i>Protonemura</i> (66), <i>Nemoura</i> (55)
Perlidae	6	18	<i>Perla</i> (8), <i>Agnetina</i> (3), <i>Dinocras</i> (3)
Perlodidae	10	62	<i>Isoperla</i> (45), <i>Besdolus</i> (4), <i>Perlodes</i> (4)
Taeniopterygidae	4	42	<i>Brachyptera</i> (22), <i>Rhabdiopteryx</i> (10)
Total	34	426	

The Asian Plecoptera

Asia lies within the Palearctic and Oriental geographic regions. With the largest continental area, Asia contains the greatest number of stonefly species with 11 families and a minimum of 1,050 species (Table 3) (Levanidova and Zhiltzova 1979, Shimizu 2001, Sivec et al. 1988, Stark 1989, Stark 1989, Teslenko 2003, Yang et al. 2004, Zhiltzova 2003, Sivec and Stark unpublished data). The Oriental realm has the richest stonefly fauna in the world (Sivec unpublished data); unfortunately, very few people are studying the region to estimate the number of species present. China alone could have between 500 to 1000 species. The Perlidae and Nemouridae families contain almost 70 percent of the species known in Asia and Scopuridae and

Styloperlidae only occur in small regions of the eastern Palearctic and Oriental (Stark unpublished data, Uchida and Maruyama 1987, Uchida and Isobe 1989).

Table 3. Summary of Asian Plecoptera.			
Family	Number of genera	Number of species	Diverse genera within family
Capniidae	~9	~ 50	<i>Apteroperla</i> , <i>Capnia</i> , <i>Isocapnia</i>
Chloroperlidae	~9	~ 30	<i>Alloperla</i> , <i>Suwallia</i> , <i>Sweltsa</i>
Leuctridae	4	~50	<i>Leuctra</i> , <i>Perlomyia</i> , <i>Rhopalopsale</i>
Nemouridae	7	~ 350	<i>Amphinemura</i> , <i>Nemoura</i> , <i>Protonemura</i>
Peltoperlidae	5	~ 50	<i>Cryptoperla</i> , <i>Peltoperlopsis</i>
Perlidae	~26	~ 380	<i>Agneta</i> , <i>Kamimuria</i> , <i>Neoperla</i>
Perlodidae	~20	~ 100	<i>Isoperla</i>
Pteronarcyidae	1	2	<i>Pteronarcys</i>
Scopuridae	1	5	<i>Scopura</i>
Styloperlidae	2	10	<i>Cerconychia</i> , <i>Styloperla</i>
Taeniopterygidae	~7	~ 20	<i>Obopteryx</i> , <i>Taenionema</i> , <i>Taeniopteryx</i>
Total	~91	~ 1047	

The South American Plecoptera

The continent of South America lies within the Neotropical realm. There are six families, 45 genera, and nearly 435 species (Table 4) present (Baumann 1982, Stark 2001a, Stark and Froehlich unpublished data). Notonemouridae and Diamphinoidea are found in Argentina and Chile, with the latter family being endemic (Orce 2003). The families Austroperlidae, Eustheniidae, and Gripopterygidae are distributed throughout the region and Perlidae, with its diverse genus, *Anacroneuria*, occurs in the northern Neotropics into the southern Nearctic realm (Stark 2001a).

Table 4. Summary of South American Plecoptera.			
Family	Number of genera	Number of species	Most diverse genera within family (number of species)
Austroperlidae	4	6	<i>Klapopteryx</i> (3), <i>Penturoperla</i> (1)
Diamphinoidea	2	5	<i>Diamphino</i> (3), <i>Diamphipnopsis</i> (2)
Eustheniidae	2	2	<i>Neuroperlopsis</i> (1), <i>Neuroperla</i> (1)
Gripopterygidae	23	~74	<i>Gripopteryx</i> (14), <i>Tupiperla</i> (12)
Notonemouridae	4	~17	<i>Austronemoura</i> (9), <i>Neofulla</i> (3)
Perlidae	10	~ 330	<i>Anacroneuria</i> (~ 280), <i>Kempnyia</i> (29)
Total	45	~434	

The African Plecoptera

Africa lies within the Afrotropical and Palearctic realms (Fig. 1). Currently, three families, 10 genera, and about 65 species (Table 5) have been reported (Baumann 1975, Stevens and Picker 1995, Picker 1980, Villet 2000). The family Nemouridae is found in North Africa

within the Palearctic realm. It's sister family, Notonemouridae, occurs in South Africa and contains 31 species (Stevens and Picker 1995, Villet 2000). The Perlidae is only represented by the genus *Neoperla*. This is the only stonefly in vast parts of sub-Saharan Africa (Picker 1980, Zwick 2000). *Neoperla* appears to be a complex of several species (Zwick 1976, Picker 1980, Sivec et al. 1988) within Africa, with earlier findings suggesting a single variable species (Hynes 1952).

Table 5. Summary of African Plecoptera.

Family	Number of genera	Number of species	Most diverse genera within family (number of species)
Nemouridae	3	~5	<i>Protonemura</i> (3), <i>Amphinemura</i> (1), <i>Nemoura</i> (1)
Notonemouridae	6	31	<i>Aphanicercella</i> (11), <i>Aphanicerca</i> (8)
Perlidae	1	~29	<i>Neoperla</i> (29)
Total	10	~65	

The Australian and New Zealand Plecoptera

Australia and New Zealand are located within the Australasian realm and are presently known to support four families, 46 genera, and about 300 species (Table 6) (Theischinger and Cardale 1987, McLellan 2003, Michaelis and Yule 2000). All of the genera and species within Australia and New Zealand are endemic to their respective country except *Notonemura* (Notonemouridae) and *Stenoperla* (Eustheniidae), which occur in both countries (Theischinger and Cardale 1987).

Table 6. Summary of Australian and New Zealand Plecoptera.

Family	Number of genera	Number of species	Most diverse genera within family (number of species)
Austroperlidae	6	11	<i>Austroheptura</i> (4), <i>Tasmanoperla</i> (2)
Eustheniidae	3	19	<i>Stenoperla</i> (9), <i>Eusthenia</i> (6)
Gripopterygidae	25	204	<i>Dinotoperla</i> (33), <i>Leptoperla</i> (28), <i>Zelandobius</i> (21)
Notonemouridae	12	61	<i>Austrocercella</i> (15), <i>Spaniocercoides</i> (8)
Total	46	295	

Summary

Sixteen families and more than 2000 species of stoneflies are recognized in the world today (Zwick 2000). They can be found on every continent, except Antarctica, and within every biogeographic realm. Diversity appears to be the greatest in Asia (~1047 species) and least diverse in Africa (~65 species). Biogeographical research and taxonomic refinement of Plecoptera needs to continue in parts of Asia (e.g. China, Borneo, Vietnam), Africa, and South America to provide a more comprehensive understanding of the order.

STONEFLY EXTERNAL MORPHOLOGY

K. Alexander

Stonefly morphological characters are ancestral to most modern insects and so they are relatively simple. Due to this ancestral morphology, understanding the structures of stoneflies not only aids in their identification and understanding of evolutionary and ecological relationships but also helps in understanding the structures of many other groups of insects.

Nymphs will be the primary focus, but some discussion of adult and egg structures will also be provided. We will progress through the tagmata (or major body regions) from the anterior end to the posterior end and the focus will be on structures that are most relevant for ecological and evolutionary relationships and in taxonomic identifications. North American families will be emphasized

Head.-The head contains the mouthparts and several major sensory structures (Figs. 3 and 4). The general shape of the head and the positioning of the compound eyes can be important. For example, the head may be elongate with the eyes set far forward or more commonly with the eyes set closer to its posterior edge as in Fig. 3. The number of ocelli (simple, single-faceted eyes on the dorsal surface) is usually three, but the perlid genera *Neoperla* (always) and *Perlinella* (ephrye sometimes) have two.

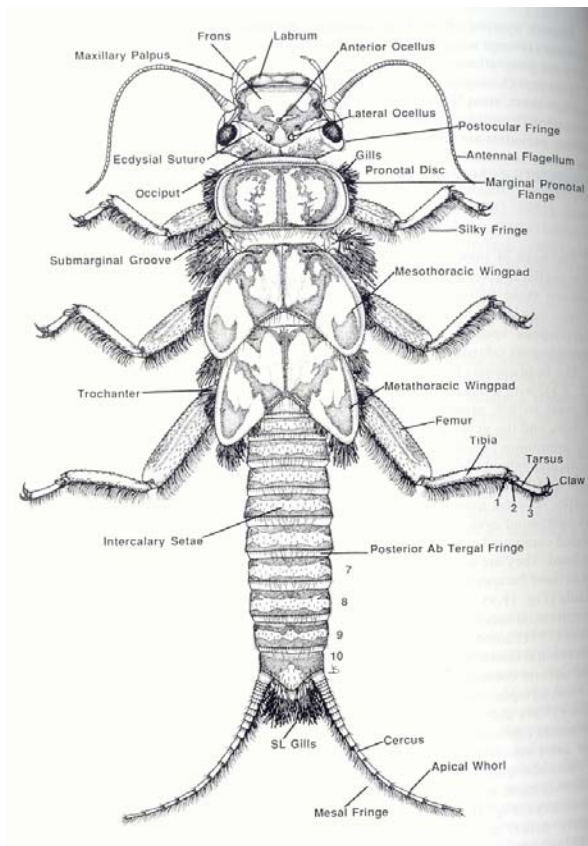


Figure 3. Nymph, dorsal view (from Stewart and Stark, 2002).

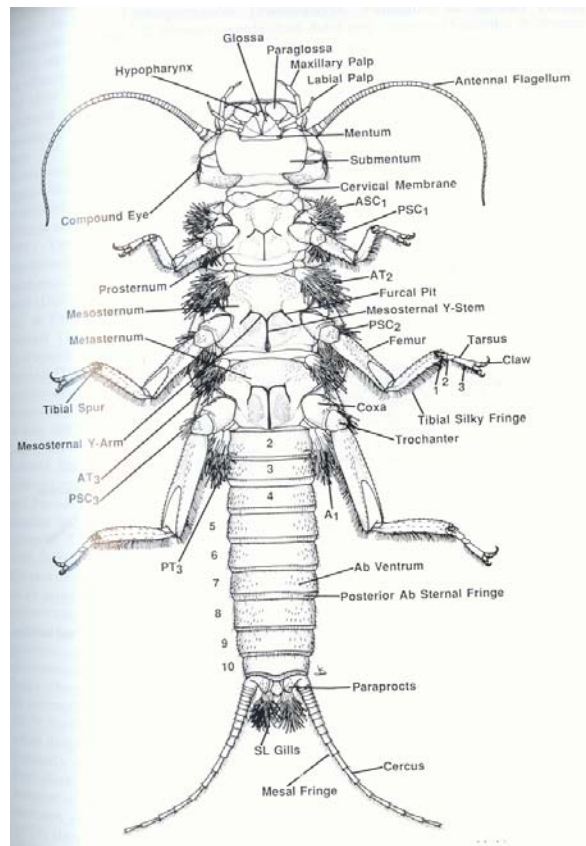


Figure 4. Nymph, ventral view (from Stewart and Stark, 2002).

The dorsal surface of the head can be variously pigmented and these pigmentation patterns, though plastic, can be diagnostic. Illustrations are often provided for these pigment patterns and usually refer to pigmentation across, between, or immediately in front of the ocelli (Fig. 5). Across the occiput (posterior edge of the head) there may be spinules (short spines) or hairs that form a row that is diagnostic in some genera of the Perlidae (Fig. 5).

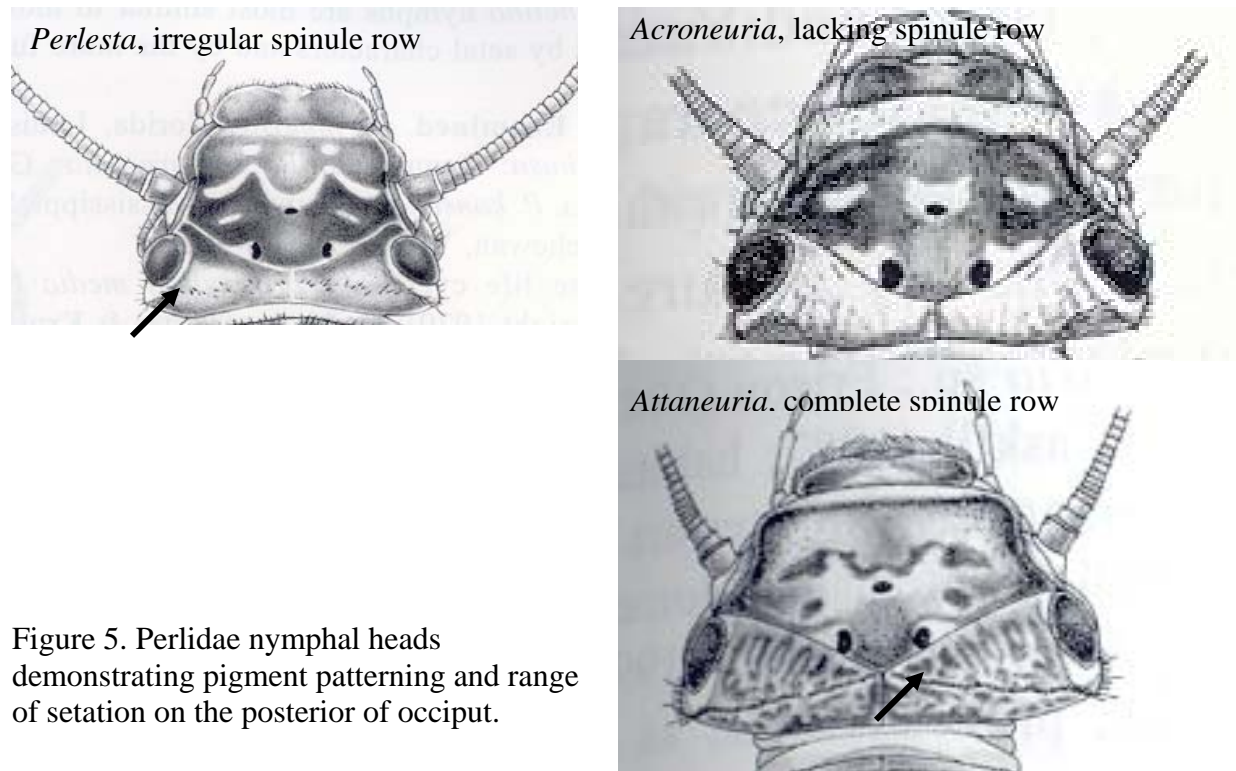


Figure 5. Perlidae nymphal heads demonstrating pigment patterning and range of setation on the posterior of occiput.

The mouthparts are tremendously useful on a variety of scales within the stoneflies and general mouthpart morphology indicates whether the taxon is primarily detritivorous (Fig. 6A) or carnivorous (Fig. 6B). The labium (“lower lip”) is composed of four lobes with the outer two lobes being termed the paraglossae and the inner two lobes termed the glossae. The Arctoperlaria is separable into the groups Euholognatha (paraglossae and glossae near equal in length) and Systellognatha (paraglossae much longer than glossae) based on the relative lengths of the two appendages (Fig. 6A&B). These two groups are largely detritivores and carnivores, respectively, but the Pteronarcyidae, Peltoperlidae and a few *Isoperla* (Perlodidae) have mouthparts that are adapted to an herbivorous lifestyle.

Mandibles for Euholognatha are usually composed of multiple, short cusps and a large molar region for grinding. Conversely, the Systellognatha have longer cusps and a reduced molar region. The most important features of the maxillae are the five-segmented palps, the lacinia, and the galea. In the Euholognatha, the galea are as large as the lacinia and bear rake or comb-like setae. The lacinia is usually armored with small truncate teeth. Both the galea and lacinia are used in feeding. The galea of systellognathans is dwarfed by the lacinia. The latter is has one or two teeth generally, with rows of hairs or hair covered bosses (bumps) medially.

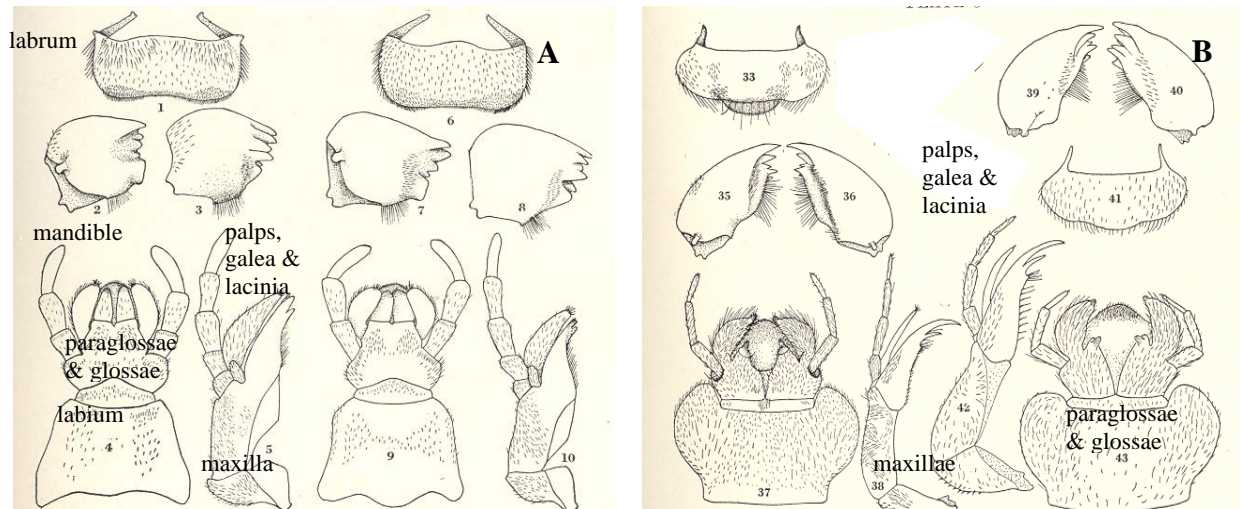
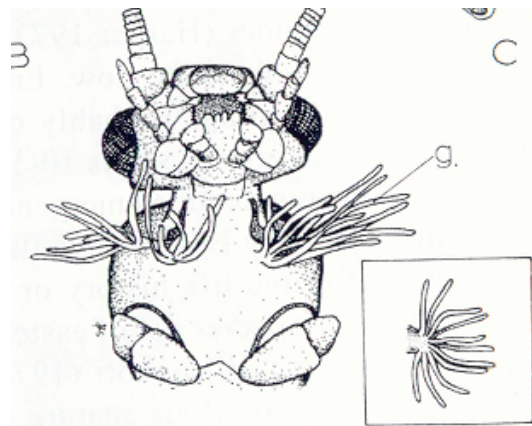
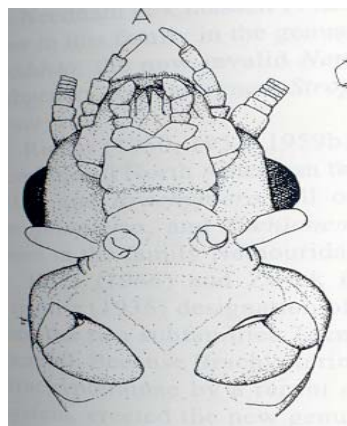


Figure 6. Mouthparts. A. Herbivorous nymphs, B. Carnivorous nymphs (from Claassen, 1931).

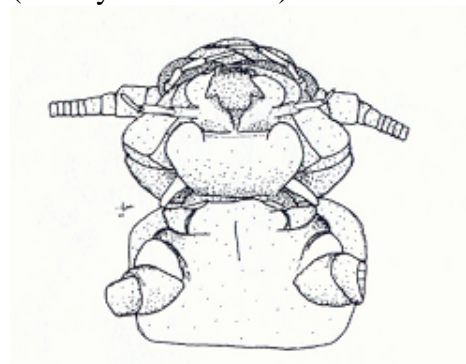
In some families, such as the Nemouridae, the positioning and shape of gills on the head are important. These gills will lie on the ventral side at the base of the labrum (lower lip) or in the cervical (neck) region. The number of branches of the gills is often important here (Fig. 7).



Branched cervical gills of *Amphinemura* (Family Nemouridae).



Unbranched cervical gills of *Zapada* (Family Nemouridae).



Unbranched premental gills of *Skwala* (Family Perlodidae).

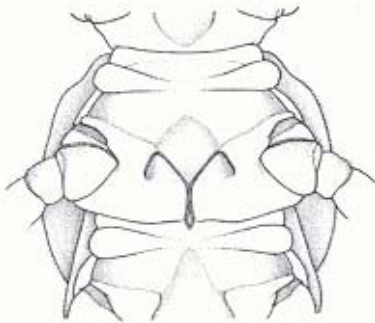
Figure 7. Stonefly gills located on or near the head (from Stewart and Stark 2002).

The antennae are long, conspicuous and anteriorly directed (Fig. 3). They are seldom used as diagnostic characteristics.

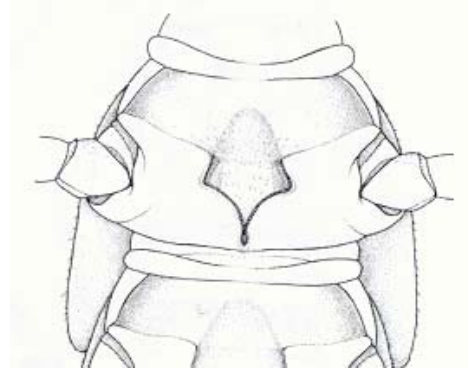
The head morphology is relevant for adults, although submental and cervical gills will be only small, shriveled remnants. The mouthparts of adult Systellognatha are reduced and presumed to be of little use for gathering food. They have not been made use of as taxonomic characters. Euholognathan adults do have functional mouthparts and many must feed on algae and lichens of trees in order to produce eggs. Taeniopterygidae have been reported to eat the blossoms of fruit and elm trees.

Thorax.-The thorax is composed of the prothorax, mesothorax and metathorax (Fig. 3 & 4). The dorsal surface of each segment is termed the notum, the sides are the pleura and the ventral surface is the sternum. The meso- and metathoracic segments have wing pads and the degree of divergence from the main body axis of fully developed metathoracic wingpads is a diagnostic character. Leuctridae and Capniidae have wingpads that do not diverge, while Taeniopterygidae, Nemouridae, Chloroperlidae, and Perlodidae wingpads diverge variably (warning: this is only valid on mature nymphs since wingpads grow with the nymph). The prothoracic segment has no wingpads but the color patterning on the pronotum (dorsal surface) is often important. Within the Perlodidae, the shape of the mesosternal Y-arms is important to identifying certain genera (Fig. 8). The Y-arms either contact the anterior or posterior ends of the furcal pits (two longitudinal furrows on the mesosternum), or the Y-arms are absent. The Peltoperlidae are distinctive in having large sternal plates that partially overlap the sternum of following segment.

Skwala, Y-arms, anterior of furcal pits



Cultus, Y-arms, posterior of furcal pits



Diploperla, Y-arms are lacking

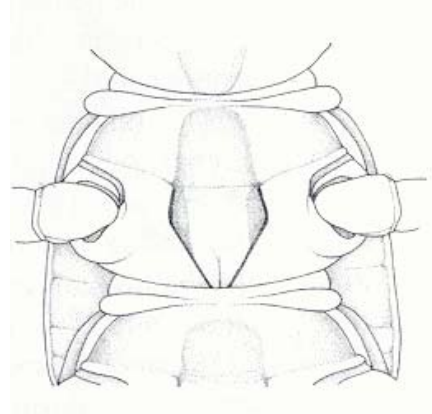
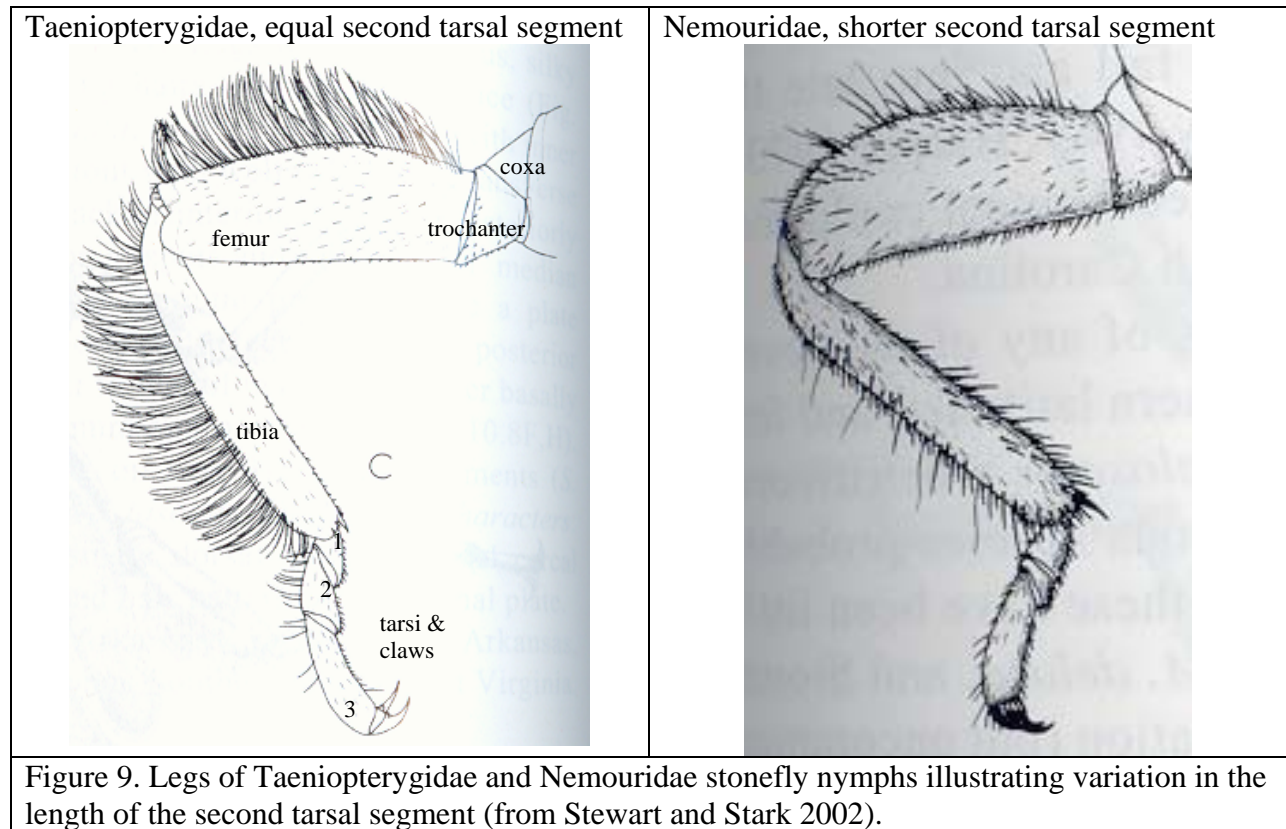


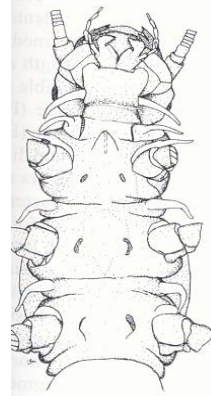
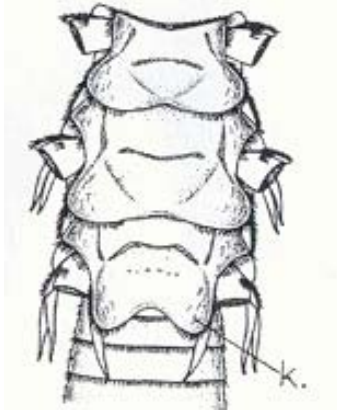
Figure 8. Mesosterna of Perlodidae nymphs (from Stewart and Stark, 2002).

The legs are composed of five regions: coxa, trochanter, femur, tibia and tarsus (Fig. 9). The extent of hairs and/or spines on the femur and tibia are diagnostic to genera in some families such as the Perlidae and Perlodidae. The tarsus is three segmented and the length of the individual segments is diagnostic, particularly to the Taeniopterygidae, which has the first and second tarsal segments approximate equal. Most other families have a second tarsal segment that is shorter than segment one.



The presence or absence of gill on the thorax and the structure of those gills, single or branched, are important characteristics in identifying families and genera (Fig. 10).

Peltoperlidae, single, unbranched thoracic gills Perlodidae, unbranched thoracic gills



Perlidae with highly branched thoracic gills

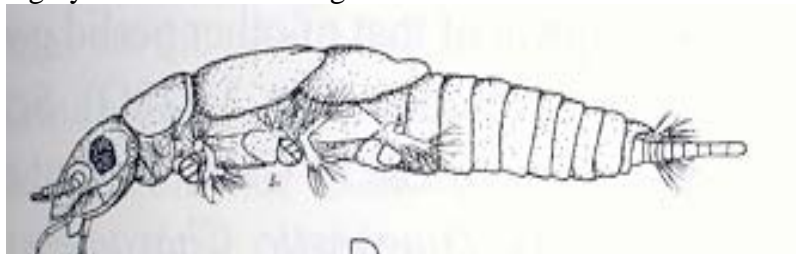


Figure 10. Variation in thoracic gills found on nymphs (from Stewart and Stark 2002).

In adults, the diagnostic structures remain virtually the same as in the nymphs, except that gills now occur as remnants and the adults possess wings full wings (macropterous), reduced wings (micropterous), or wings are absent (apterous). The wings occur on both the meso- and metathoracic segments with the hind wing usually being broadly expanded in the anal vein region (see below) and larger than the forewing. The wings have a series of longitudinal veins (Fig. 11). These veins, from leading edge to trailing edge, are: costa, subcosta, radius, median, cubitus, and anal. Variation in wing venation is complex and requires much explanation that is beyond the scope of this workshop. Most diagnostic characters in wing venation involve the presence and positioning of veins and crossveins. It is best to reference illustrations within the specific key you are using to identify these.

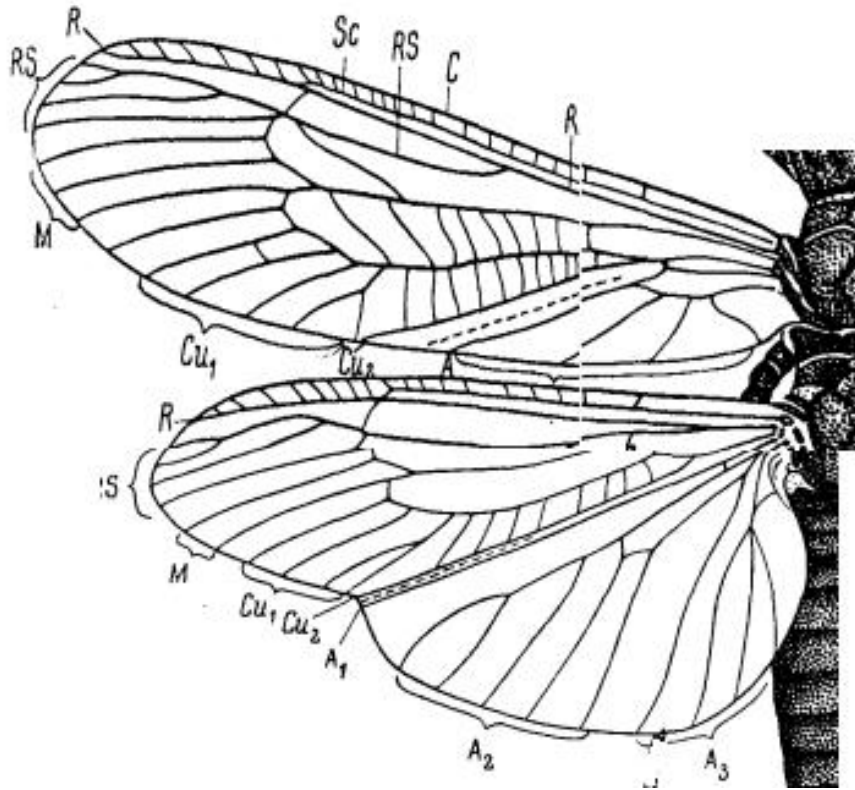


Figure 11. Wings of an adult stonefly showing generalized venation.

Abdomen

The abdomen is composed of 10 segments and, similar to the thorax, the dorsal surface of each segment is termed the tergum (=notum on thorax), the sides are the pleura and the ventral surface is the sternum (Figure 3 & 4). The segments that have a distinct pleural region, seen as a fold, vary from four to a maximum of nine (Capniidae only); thus, this is a diagnostic character to family and genus in some groups (warning: this is difficult to see, especially in poorly preserved specimens). The anus lies ventral to the tip of the tergum 10.

The color patterning on the dorsal surface varies by species as does the abundance, arrangement, and structure of setation (hairs). This variability is useful to identify genera within a few families.

In Nearctic stoneflies, gills occur on the abdomen only in the families Pteronarcyidae (abdominal segments one through two or three), Perlidae (near the anus at the posterior end of the abdomen) (Figure 3 & 4) and on the rare western North American perlodid genus *Oroperla* (abdominal segments one to seven) (Fig. 12).

Pteronarcella (Pteronarcyidae) with gills on abdominal segments 1 to 3



Oroperla (Perlodidae) with gills on abdominal segments 1 to 7

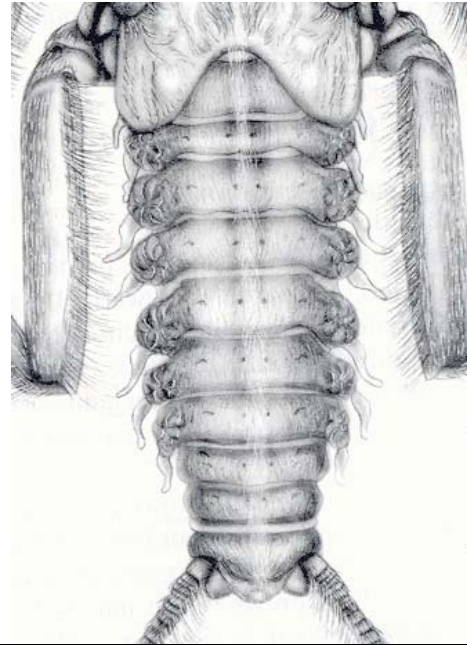
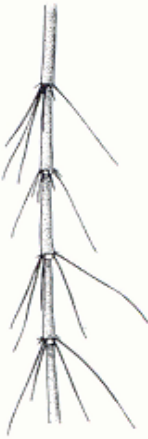




Figure 12. Illustrations of stoneflies with gills on the abdomen (from Stewart and Stark, 2002).

The cerci (“tails”) number two in stoneflies, as opposed to three (with one anal filament and two cerci) as in many mayflies. The cerci are long and whip-like and vary in length from much longer than the abdomen to less than $2/3$ the length of abdomen. In adults, the cerci can be reduced to a single segment. The number and position of hairs on the cerci is also variable and is an important diagnostic character in some nymphs. For example, the length of hairs at the posterior end of each cercal segment, dorsal row of hairs, and the presence intercalary hairs (on the cercal segment itself and not at the tip) are all diagnostic to various taxa (Fig. 13).

<p>Apical section of cercus from <i>Perlinella</i> (Family Perlidae) showing long hairs at apex of each segment.</p> 	<p>Apical section of cercus from <i>Perlinodes</i> (Family Perlodidae) showing dorsal fringe of hairs.</p> 
<p>Apical section of cercus from <i>Attaneuria</i> (Family Perlidae) showing intercalary hairs.</p> 	
<p>Figure 13. Cerci of stoneflies illustrating differing hair characteristics (from Stewart and Stark, 2002).</p>	

Adult stoneflies have internal and external reproductive structures that are diagnostic at both higher levels and for species. Male structures are by far the most taxonomically important ones—they contain structures with the greatest specificity (Fig. 14). Many stoneflies have a reflexed appendage called an epiproct that can be highly variable between species (Fig. 14A). It is used to pry down the female's subgenital plate (arising from the eighth abdominal sternite) (Fig. 14B) and to transfer sperm. Males may also have paraprocts that may or may not be modified for prying the female's plate (Fig. 14.C). In this format, paraprocts help to bring into apposition an extruded, internal reproductive structure called an aedeagus. This structure transmits sperm and often has spinule patches, sclerites, and caecae that are species specific. To be used as a taxonomic character, it must be extruded when the stonefly is alive or delicately

everted after death. The Peltoperlidae, Perlodidae and Perlidae have such paraprocts and an aedeagus. Some genera of Perlidae have hooks developed from divided lobes of the tenth tergite, the hemitergal lobes (Fig. 14E). Here the epiproct is reduced, the paraprocts are unmodified, and an aedeagus is present. Stonefly males in the Arctoperlaria may also have structures on the abdominal sternites that are used to produce substrate born vibrations for mate location (“drumming”) (Fig. D & F), although there are some species that drum which have no such structures. The presence, location and morphology of these drumming structures can also be family, genus, or species specific.

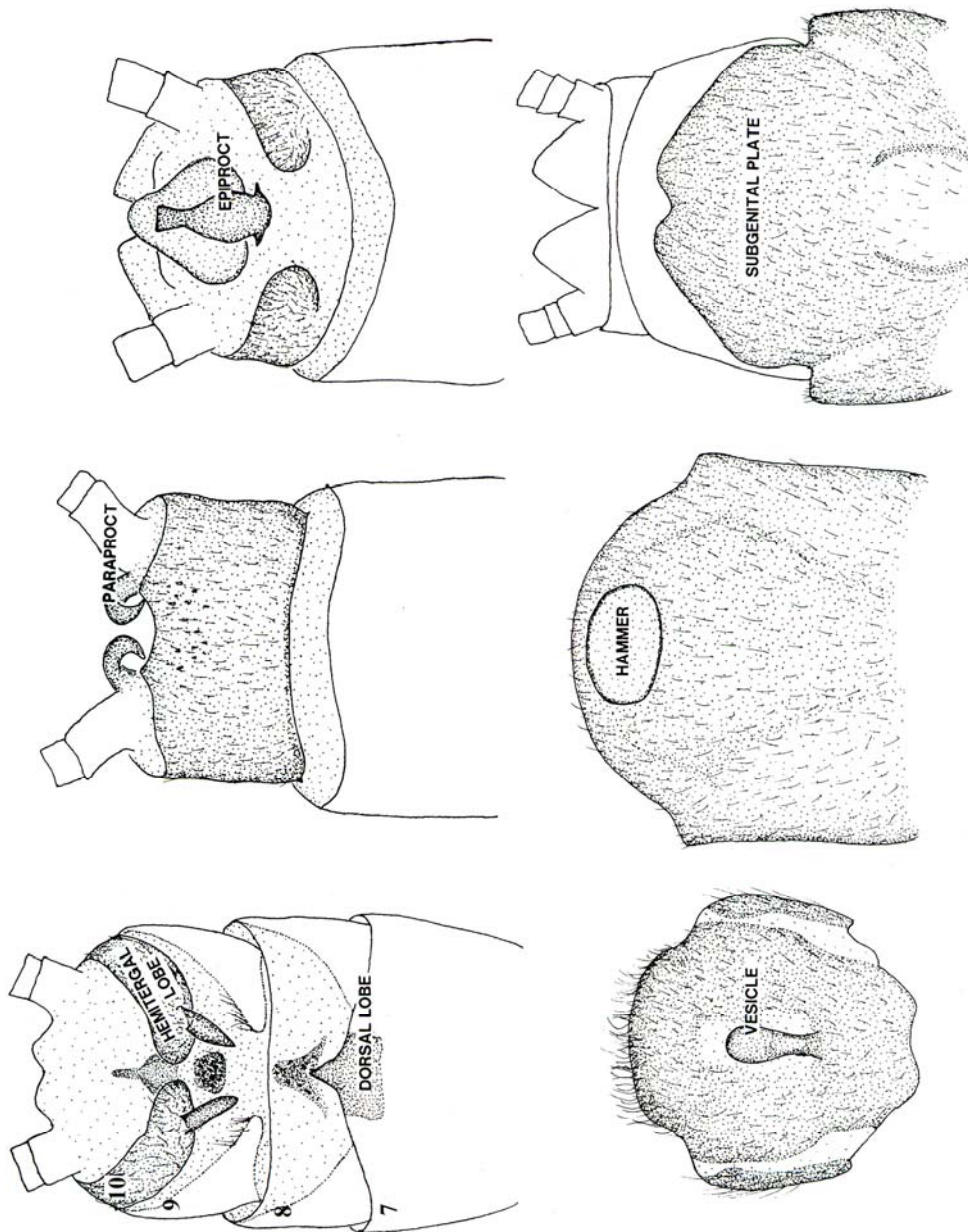


Fig. 14. External reproductive structures found on male and female stoneflies. From Stark et al. 1998.

Eggs.-Stonefly eggs vary tremendously in shape, size, and structure. While most stoneflies have eggs with a sclerotized chorion, the Euholognatha have membranous eggs. The chorion of sclerotized eggs can be smooth or studded, have follicular cell impressions, and have bizarre ridges. Eggs may also have collars that are tall, short, narrow, wide, or absent. Micropyles provide guides for sperm to fertilize the eggs. These egg features are used in combination with male and female external and internal genitalia to make species determinations.

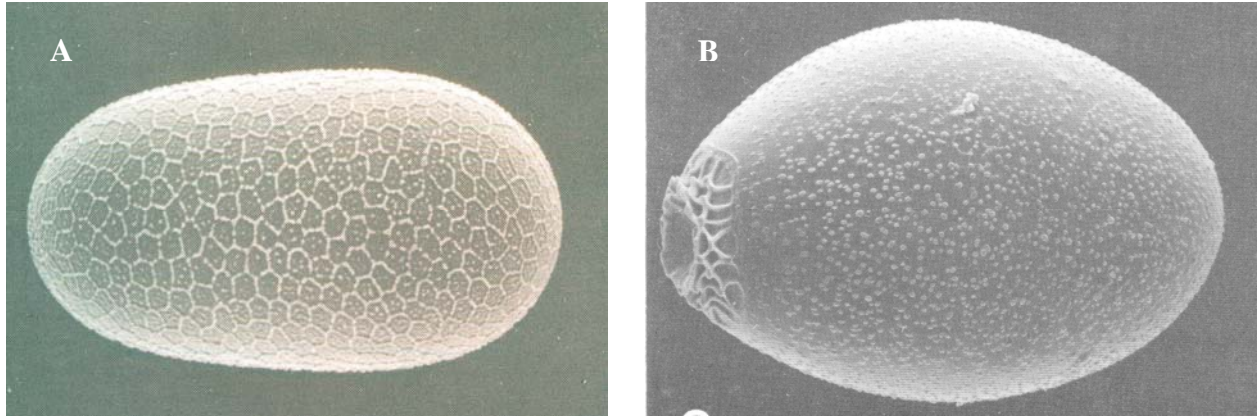


Fig. 15. Eggs of two Chloroperlidae. A. *Suwallia sierra* Alexander and Stewart, B. *Suwallia wardi* Kondratieff and Kirchner. Note the presence of an egg collar and the lack of follicular cell impressions on B. From Alexander and Stewart 1999.

KEYS TO ADULTS AND NYMPHS OF NORTH AMERICAN STONEFLY FAMILIES

Stark et al. (1998) produced an easy to use, pictorial key to stonefly families in North America. It includes the adults and nymphs in three well illustrated panels, with a minimum of text. This format has been used successfully elsewhere for aquatic insects (McCafferty 1981), and it has several advantages over traditional keys. We will walk you through the use of this key, allow you to practice for about an hour using the specimens we have provided. There will be a short quiz before lunch to give us an idea of how well you are doing.

ADULT STONEFLY KEY

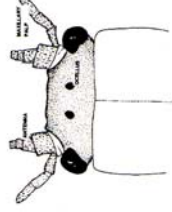
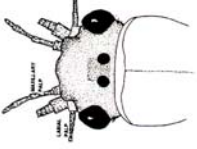
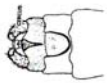
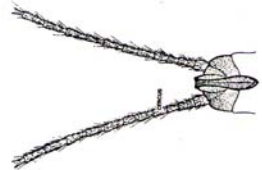
Basal tarsal segment
subequal to, or longer
than, apical segment

Basal tarsal segment
much shorter than
apical segment



Mid tarsal segment
subequal to basal
segment
TAenioPTERYGIDAE

Mid tarsal segment
small and wedge
shaped

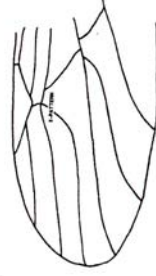
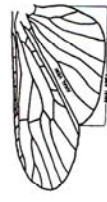


Cerci multisegmented
CAPNIIDAE

Cerci 1-segmented

Labial palps project
beyond margins of
head, visible dorsally;
2 or 3 ocelli

Labial palps not visible
in dorsal aspect, 2 ocelli
PTELPERLIDAE



Wings rolled around
body at rest; no X-
pattern on forewings
LEUCOTRIDAE

Wings flat at rest;
anteroapical forewing
veins form an X-pattern
NEMOURIDAE

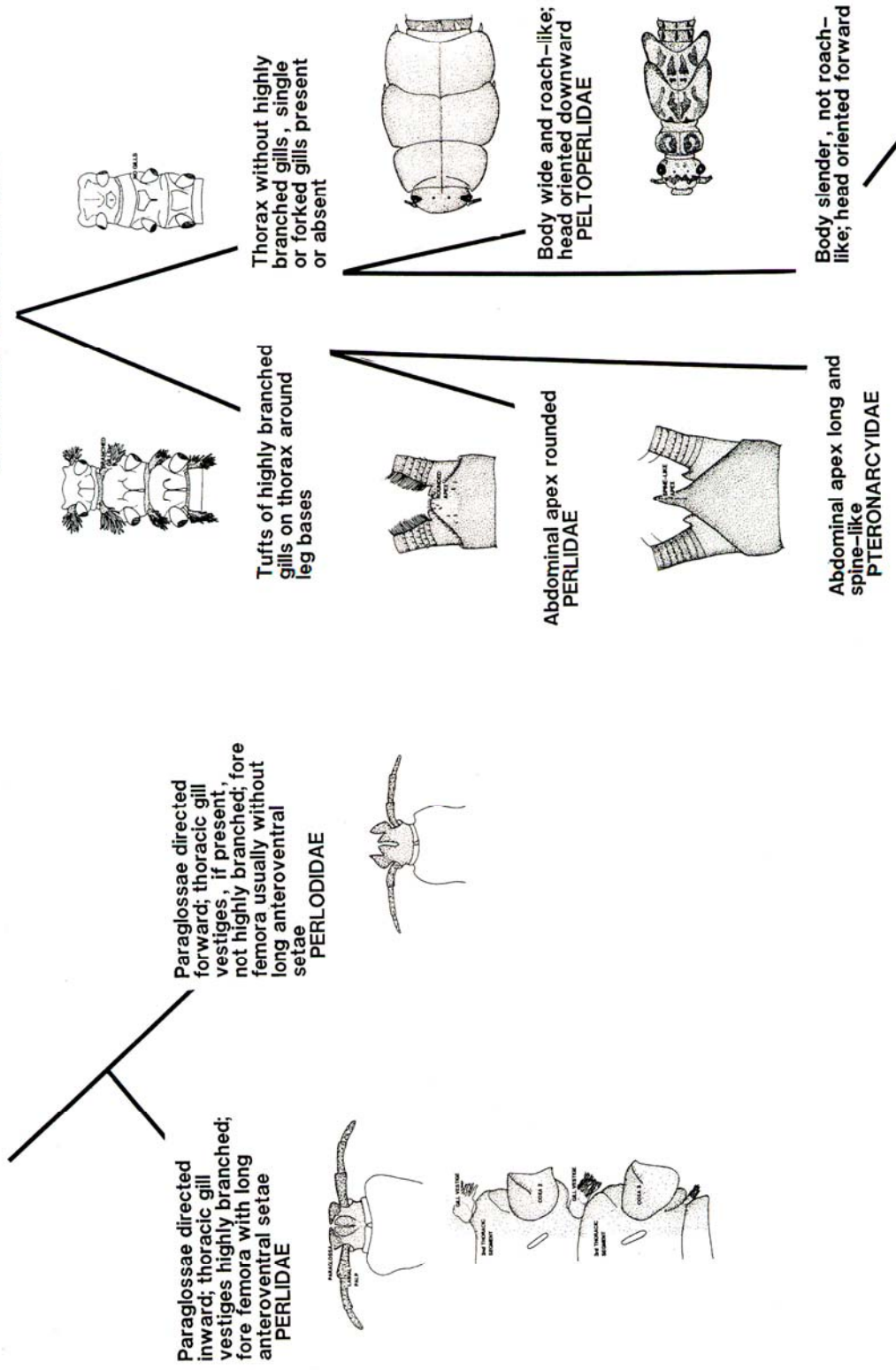
Anal area of hindwing
broad, usually with 5
or more anal veins

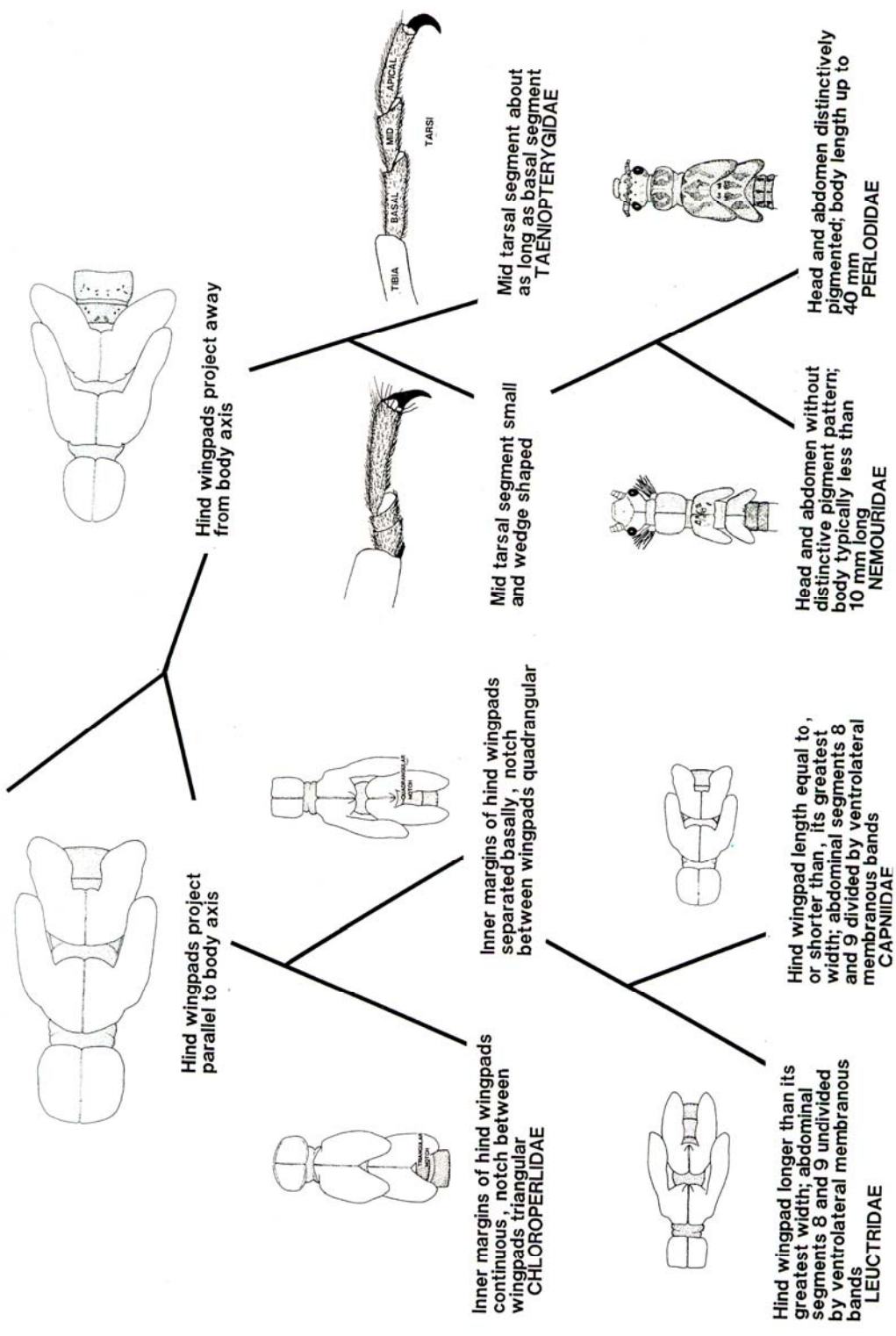
Anal area of hindwing
narrow, usually with
less than 5 anal veins
CHLOROPERLIDAE

Forewing anal area
with 2 or more rows
of crossveins
PTERONARCYIDAE

Forewing anal area
with, at most, one
row of crossveins

NYMPHAL STONEFLY KEY





QUIZ

	Your Answer	Our Answer	Comments
1			
2			
3			
Number Right:			

LIFE HISTORY AND ECOLOGY OF STONEFLIES

Barry Poulton

Life History Traits

Metamorphosis.-The order Plecoptera is considered by many to have a hemimetabolous (incomplete metamorphosis) type of development, because no pupal stage exists and the nymphs and winged adults normally live in different media (aquatic vs. terrestrial). Recently, some entomologists have considered the Plecoptera as paurometabolous (a type of gradual metamorphosis) because nymphs have gradual, external wingpad development and adults usually resemble the nymphs in body shape and form (Stewart and Stark 2002). Stonefly nymphs molt periodically throughout their life cycle, with about 12-23 instars known for most species (Hynes 2000). They are either univoltine (one generation per year) or semivoltine (one generation every 2 or 3 years). Only one species, *Nemurella pictetii* (Nemouridae), from Europe, has been confirmed to finish more than one generation per year (Wolf and Zwick 1989). Astonishingly, it has been shown to have partial bivoltine and trivoltine life cycles from different populations.

Life cycle types.- Life cycle types are defined based on the length of time between the onset of egg hatching and emergence of the adult stage. Species with “slow” life cycles have eggs that hatch shortly after being laid (immediately or within 2-4 weeks) and have gradual nymphal growth (Stewart and Stark 2002). This is typical of such stoneflies as *Neoperla* (univoltine-slow) or some *Acroneuria* (some are semivoltine-slow, both Perlidae). Species with “fast” life cycles have eggs or early instar nymphs with a prolonged period of dormancy (diapause), followed by rapid nymphal growth when exposed to specific environmental conditions.

Several winter-emerging species (e.g., Capniidae: *Allocapnia*; Taeniopterygidae: *Taeniopteryx*) have fast life cycles with eggs hatching soon after being laid. Interestingly, some *Allocapnia* nymphs migrate deep into sediment and become dormant until fall when they resume a rapid growth for several months, demonstrating a nymphal diapause (Pugsley and Hynes 1985). Some semivoltine species, for example *Isogenoides zionensis* (Perlodidae), have an approximately nine-month egg diapause followed by a year of nymphal growth (DeWalt and Stewart 1995). This egg diapause establishes this species as having a semivoltine-fast life cycle. Diapause seems to allow species that use it the ability to avoid unfavorable summer conditions. That this trait is present in populations that reside in cold, alpine streams suggests that the species, or its ancestor, had experienced poor water quality conditions in its evolutionary past.

Nymphal Growth and Development- Nymphal growth is incremental and its rate and length dependent on the life cycle type of the species. Water temperature is one of the most important factors controlling the rate of growth and development. Some species have a great deal of flexibility in relation to seasonal differences, whereas others are only known from habitats where annual differences in temperature are relatively minimal. Behavioral drift rates in Plecoptera nymphs are relatively low, but in some species, drift increases as development approaches emergence; this has been observed most often with territorial species, where drift is thought to provide relief from population overcrowding.

Adult Emergence and Mating.-Emergence of Plecoptera species requires the right cues related to temperature, day length, and the distribution of growth hormones. Emergence of a generation

may occur rapidly within a short time period (synchronous), may be staggered out over several weeks (extended), or may take place at nearly any time of the year (asynchronous, a few species in spring habitats) (DeWalt and Stewart 1995). Nymphs migrate to the stream margins or suitable mid-water habitat, crawl out of the water, and cling to a solid surface. This last nymphal stadium ends when the nymphal skin splits atop the thoracic nota and the teneral adult pulls out of the exuvium. Adults attain their permanent coloration after several hours, at which time the final exoskeleton has dried and hardened.

Wing length varies among species and one may infer relative wing length by inspecting wing pad development of last instar nymphs (these will often have darkened wingpads). Those with abbreviated wing pads will produce shorter wings. Macropterous wings are generally slightly longer than the abdomen, brachypterous or micropterous wings have completely developed but are somewhat shorter than the abdomen, and those with wings highly reduced or absent as said to be apterous.

Adult longevity ranges from several days for some winter emerging species, to a couple of weeks for summer species (DeWalt and Stewart 1995). Many of the Euholognatha feed as adults, scraping fungal/algal/lichen coverings off trees and other substrates (Frison 1929). Taeniopterygidae have been observed to eat the buds and flowers of riparian trees and have been suggested to damage fruit tree blossoms (Newcomer 1918). However, it appears that most Systellognatha do not feed. Several species in this group emerge with fully formed eggs; however, many require one to several days to produce fully sclerotized eggs. It is possible that some feeding occurs in some Systellognatha, even though their mouthparts appear to be of little use. Stoneflies have been observed to consume water and plant nectar, which presumably serves to prevent dehydration and increases longevity.

After emergence, adults are often inconspicuous and hide in streamside vegetation or debris. However, large, highly visible hatches have been observed in some species. Adult winter stoneflies in the families Capniidae and Taeniopterygidae are among the most active and diverse groups of insects in winter months. This seems counterintuitive, since they are often observed running over snow at temperatures just under freezing; however, they all have a dark coloration and an antifreeze substance in their body fluids to resist the effects of below freezing temperatures.

During warm seasons, adults may take flight shortly after emergence. Species that emerge during winter or early spring crawl for considerable distances, often on vertical surfaces such as trees or bridges until they reach an elevated position that allows maximum exposure to sunlight. Capniidae can often be seen skating across the top of streams, using their upturned abdomen and wings as a sail (DeWalt unpublished data). Taeniopterygidae will fly if temperatures are mild.

Many stoneflies utilize a communication system that consists of tapping or rubbing the abdomen on substrates (drumming) to locate mates. Males initiate the communication and females answer and remain stationary while the male searches. This “duet” eventually leads the male to an ever smaller, restricted search of substrates for the female. A vast diversity of simple beats, bimodal signals, stridulation, and tremulation has been observed in the Arctoperlaria. The number of beats, beat intervals, and beat configurations is species-specific. Ken Stewart, a longtime member and past president of NABS, has pioneered the recording of drumming and has discussed the evolutionary significance of the various patterns and structures associated with drumming.

Dispersal of winged adults varies widely among species; some remain close to the stream during their entire adult life, whereas summer species are often collected at lights several miles from where they emerged. Oviposition is accomplished by flying over the waterbody and dropping an egg mass from height, by flying to the stream surface to deposit the eggs, and by crawling to the waters edge and dipping the abdomen and egg batch into the water. *Megaleuctra* (Leuctridae) has an ovipositor and so may have another type of oviposition not described here. Stoneflies are rather poor fliers generally, so they often use riparian vegetation to gain height before flying to the stream for oviposition. While a height advantage is gained, they expose themselves to greater predation by birds, bats, and other predators.

Eggs and Egg Hatching.- Oviposition of eggs can occur immediately after mating, or after a period of egg maturation. Eggs of most species are extruded at the tip of the abdomen and deposited as a cluster held together by an adhesive substance. Some species deposit multiple egg batches during their adult life, possibly spreading reproductive risk across multiple time frames and watercourses. Eggs, upon entering water, hydrate and separate from the batch, adhering to a wide range of substrates. Hatching, or eclosion, of the first instar nymph is accomplished by using the frons or an egg tooth to push open the chorion.

Adaptations

Stoneflies as a group have adapted to a wide range of environmental conditions. For many species, the onset of egg hatching and growth coincides with the autumn pulse of leaf litter entering the system. In streams and rivers with high stonefly diversity, emergence of at least one species may occur every month of the year. Closely related species have adapted to coexistence in the same stream by timing their nymphal growth and emergence periods in a staggered fashions, presumably to avoid competition with other species. As many as 30-40 species of stoneflies can be found in lotic systems that have excellent water quality and habitat diversity. Species that are present in temporary or intermittent streams usually have heterodynamic life cycles, where a period of egg or nymphal dormancy exists. Diapause of eggs or nymphs is interrupted by changes in stream conditions (re-hydration of the stream bed, temperature changes) that cause egg hatching or increased nymphal growth rates. Viable eggs of stoneflies have been recovered from streambed sediments that have been dry for up to a year.

Value to Fish and Wildlife

Stoneflies provide a valuable food source for a wide variety of vertebrates. Nymph and adult stages are eaten by many species of fish and amphibians. Trout are known to feed selectively on drifting stonefly nymphs, and feed heavily on winged adults during well-known hatches such as that of the Western Salmonfly (*Pteronarcys californica*). Large river, benthic fishes, such as sturgeon, forage extensively on stonefly nymphs during certain times of the year. For bats and birds such as Nighthawks that feed at dusk on flying insects, actively dispersing winged adults are a valued and plentiful food source during warm seasons. Though not well documented, migrating birds find a plentiful supply of winter and early spring stoneflies. Stonefly nymphs and emerging adults are also eaten by mammals such as shrews, raccoons, and mink.

Ecological Requirements

Stoneflies have the ability to colonize nearly all habitats and substrate types available in streams and rivers. Nymphs of most species require flowing water, but a few species are known to occur

along the wave-swept shores of large water bodies or cold snowmelt lakes in mountainous altitudes. High dissolved oxygen is required for most species and these oxygen requirements are more critical in situations where water movement does not exist. A few species can be found in splash zones of waterfalls or thin films of moisture created by seeps that flow over vertical rock surfaces. Some (*Soyedina*: Nemouridae) live in organic seeps where no perceptible flow can be detected. Still others, in some Australian Antartoperlaria, can accomplish a life cycle with little moisture at all. No stoneflies live in marine environments.

Water Temperature

Many stonefly species require a specific range in annual maximum and minimum water temperatures, because life history traits such as egg hatching, growth, and emergence are usually temperature-dependent. Stoneflies are often absent below impoundments with hypolimnetic-release dams because annual temperature fluctuations in these systems have been artificially altered and do not match the thermal cues to which they have adapted. In the Ozark region, the thermal regime of some streams is naturally modified by high-volume springs, resulting in lower summer maximums and higher winter minimums. In these instances, disjunct populations of stonefly species that are associated with cooler streams in more northern latitudes may be present. Due in part to these phenomena, stonefly distributions are valuable for zoogeographic studies, and are key indicators of past dispersal during glacial periods. This has been aptly demonstrated by Ross and Ricker (1971).

Food Availability and Utilization

Stonefly nymphs are classified into several functional feeding groups, and are shredders, herbivore-detritivores, or predators. However, a few groups of stoneflies (some *Isoperla*: Perlodidae, for instance) are known to be omnivorous and will change their food habits from herbivory to a predaceous existence as nymphal growth progresses (Stewart and Stark 2002). Some nymphs are shredders of leaf material and this is a convenient feature for the rearing of these species. In addition to the labile organic compounds in leaf material, shredders and herbivores also ingest the fungal and bacterial colonies associated with leaf litter. Food preferences and selectivity have been determined for only a few species.

Predatory stoneflies usually engulf their prey whole. Due to adaptation in feeding types, the variety of strategies observed in stoneflies as a group is evident in good quality streams, where at least one species may occupy nearly every microhabitat available. In habitats such as leaf litter accumulations and coarse mineral substrates, both herbivores and predators can be found. Predatory stonefly species are commonly found in leaf packs because their prey items are also found there.

HABITAT QUALITY AND SPECIES CONSERVATION

R. Edward DeWalt, Barry C. Poulton

Stoneflies are one of the most sensitive indicators of stream quality (see Hilsenhoff 1987 for tolerances to organic pollution). Some species are ubiquitous within a region and are considered generalists because they can survive in a wide variety of streams. Often, these species have fast, heterodynamic life cycles that provide a narrowed nymphal growth period, opposite the worst water quality conditions.

Other species have very specific requirements and are found as isolated, widely scattered populations in unique watersheds, stream types, or habitats. These species are normally the most sensitive to disturbance, and are the first ones to disappear from a region because of anthropogenic impacts. They are also the most difficult to replace; elimination of a species within an isolated watershed may not be recovered by dispersal because populations are often isolated by great distances. Zwick (1992) discussed the degradation of streams in Europe, where the topography and cultural practices left high elevation and topographically diverse regions as refugia for aquatic species (including stoneflies) and largest river systems were the most heavily degraded. This picture holds in parts of North America of similar topography and cultural practice, but breaks down in the glaciated Midwest. Here, agricultural practices and the flat topography combine to eliminate, or severely impact, the smallest streams in a drainage (Mattingly et al. 1993, DeWalt et al. accepted).

The riparian zone is important for stoneflies because in most cases it provides much needed organic matter (tree leaves, primarily) that support prey and predator populations. It also provides important emergence habitat, shelter from predators, and a staging area for successful reproduction. The riparian zone also provides a buffer from sources of stream degradation such as erosion and non-point source runoff. In general, stoneflies cannot tolerate poor water quality caused by excessive nutrient enrichment and siltation, both of which are directly responsible for increasing substrate embeddedness, reducing interstitial space. The same factors also decrease the quality of organic substrates such as leaf litter accumulations and root mats.

Stream channel alterations have a tremendous negative impact on stonefly assemblages. Chief among these is channelization, the digging of straighter channels with uniform bottom characteristics. This is accompanied by removal of natural riparian vegetation and tiling (laying of long tiles into agricultural fields to drain field faster). Other results include dramatically altered hydrologic regime (with little or no hydraulic refugia), greater fluctuation in water temperatures, and stagnant flows by late summer (Wiley et al 1990). This leaves a very few generalist stonefly species (having egg or nymphal diapause) inhabiting channelized streams. If streams cannot be adequately buffered or otherwise protected from the effects of these activities, the elimination of most stonefly species usually occurs before the disappearance of other aquatic insect groups such as caddisflies (Trichoptera), mayflies (Ephemeroptera), or Diptera.

Habitat improvement in degraded segments of streams can greatly increase the chance of community recovery from outside sources of colonization (dispersal, upstream and downstream emigration, etc.). These activities include bank stabilization, restoration of riparian buffer strips, creation of wetlands in headwaters, re-meandering of channels, and addition of hard substrates that increase hydrologic diversity or interstitial space. Stream habitat improvement is not complete restoration, since the community is still missing, but it improves recovery potential. These activities, completed on a broad scale, with the aim of increasing connectivity of

watersheds, provide an increased dispersal probability between corridors. However, the most sensitive of species, or those with isolated distributions, often cannot be re-established after they have been eliminated, even after watersheds have experienced dramatic improvement. Therefore, the most effective way to conserve stonefly species diversity is to buffer and protect high quality streams from being further degraded.

Presently, the conservation status of stoneflies is not well known, but evidence suggests that the order is in trouble in many areas. Master et al. (2000) suggest that stoneflies are the third most imperiled group of aquatic organisms in the USA. It is difficult to know just how accurate this assertion is, because the available data are scattered in the literature and in multiple natural history collections and the historical record of what occurred and where it was is not detailed enough.

The Illinois Natural History Survey has the best record of historical distributions of stoneflies, and a history of stonefly researchers throughout the 20th Century. It is thought that about 28% of the 76 species known to occur at one time in Illinois no longer reside there. This includes two extinctions of endemics (*Isoperla conspicua*, and *Alloperla roberti*) and 19 extirpations. Of the remaining species, 24% are presently known from five or fewer locations—this includes species that once had wide distributions in the state. Some areas of Illinois have maintained their historic assemblage of stoneflies, while others (heavily agricultural, urbanizing, and large river areas) have seen dramatic declines (DeWalt et al. accepted). One should hope that Illinois is not representative of its neighboring states, but this is not the current working hypothesis.

Natural history collections, museums, and the private collections of aquatic entomologists have the best data on historical and contemporary stonefly distributions (and other taxa as well). Their data are backed up by specimens, which can be re-evaluated when revisions are published. Plecopterologists need to compile these data into a single or distributed system system to aid in the conservation of stoneflies. We also need to have the cooperation of researchers whose samples contain stoneflies to deposit material in the collections of accredited institutions. It is obvious that as a community, Plecopterologists cannot accept all this material, verify its identity, and curate it without careful consideration to the provision of minimal resources for its inclusion into collections. Perhaps deposition of voucher specimens should be aspired to by professional societies such as NABS. This is not an indictment of the present system, but a quest for a dialogue.

METHODS FOR COLLECTION AND REARING OF STONEFLIES

Barry C. Poulton

Nymph Collection.-Methods for the collection of stoneflies may be reviewed in Stewart and Stark (2002), Frison (1935), and Hitchcock (1974). Largely, the same methods utilized for many other insects found in flowing waters are also effective for collecting stonefly nymphs, but what is presented here is from two decades of field experience. Qualitative devices such as the D-framed or rectangular-framed nets fitted with a handle, and kick seines or screens that are attached between two poles, are most commonly used. In riffle areas, substrate at the upstream end of the device is disturbed by kicking or shuffling, and dislodged organisms are carried by current flow into the net. Quantitative samplers used in streams include square or cylindrical devices that enclose a known area of substrate such as the Surber, Hess, or Brown. Substrate is disturbed with the use of a hand rake or claw, and organisms are carried into an attached net either passively or with a vacuum suction device. In habitats where current flow is not sufficient to transport dislodged organisms, D-framed nets can also be used to actively sweep through areas of substrate. Nymphs that are part of the behavioral drift in streams can be collected with net and frame devices that are attached to a harness or in-stream apparatus, and anchored for extended periods. Nymphs can also be collected with the use of hand methods, by turning over rocks and handpicking through leaf packs or debris accumulations, and removing nymphs with a forceps. Suitable preservatives for stonefly nymphs include 75-80% ethanol or isopropanol, 5% buffered formalin, and Kahle's solution. The latter solutions negate the use of these specimens for conservation genetics or molecular systematics studies. It is advisable to indicated the fixative history if you voucher material with an accredited museum.

Some species cannot be collected readily because their preferred habitats are not accessible with standard gear types. In the deep water of larger streams and rivers, artificial substrates such as baskets filled with rock or Hester-Dendy plates, are often colonized by stonefly nymphs. In some cases, fisheries gear such as benthic trawls or beam trawls can be used to collect nymphs if they are fitted with mesh sizes that are small enough for invertebrates. Grab samplers that are operated in a vertical fashion, will occasionally collect stonefly nymphs, but normally these devices are not used in flowing water areas. Perhaps an unique example of a difficult-to-reach stonefly habitat is that of the Flathead River in Montana, where the hyporheic zone extends deep below the streambed and laterally a considerable distance away from the stream channel. Because nymphs of some *Paraperla*, other Chloroperlidae, and *Isocapnia* (Capniidae) prefer this habitat, they are most easily collected incidentally from water supply wells located within the floodplain.

Collection of Winged Adults.-Both active and passive methods can be used to collect winged adults. Active methods include devices that are operated in or near the riparian zone, and include sweep net, aerial net, and beating sheet. Both the sweep net and beating sheet will collect stoneflies from streamside vegetation. The sweep net is brushed through branches, leaves, weedy shrubs or grasses to capture adults that are hiding in these areas. The beating sheet is held below streamside vegetation, and adults fall onto the sheet surface when branches or shrubs are shaken or disturbed and is most effectively used when air temperatures are cool. An aspirator is often used in conjunction with these methods, where specimens are sucked from the net or sheet into a vacuum tube before preservation. An aspirator is most useful for winter species, in which

large numbers of adults can be efficiently removed from the surfaces of bridges or other objects. The aerial net is utilized when adults are actively flying. In cooler climes, this usually takes place after a couple hours of sunshine. Flights also have been observed in late afternoon, where presumably the confused diurnal adults leave dark forest edges and fly to openings.

Winter stoneflies (Capniidae, Taeniopterygidae, some Leuctridae and Nemouridae as well) emerge throughout the winter. They can be collected on mild (above freezing), sunny days from bridges and from snow and ice at the margin of streams. However, not all winter days are like this and it may be that you have to collect when temperatures are below freezing. The adults are still there and may be collected by looking in sheltered habitats such as dry debris piles (leaves, grass hummocks, drift line of flood residue) and the bark of trees. In cold weather, a beating sheet is the best gear, although one may substitute an old umbrella. Shake the residue on to this surface and remove the debris. Hopefully, your beating sheet will be filled with *Allopcania* and *Taeniopteryx*.

Passive methods include devices that are placed in or near the stream to attract flying and crawling adults. The most commonly used device for adults during warm seasons is the ultraviolet light, which can be mounted on a white sheet for hand picking. This allows for careful collecting of adults from the sheet if only one sex or select species are desired. Often, researchers are after males that require extrusion of the aedeagus for proper identification, an activity facilitated by the sheet. If your research depends on species identification for such genera as *Perlesta*, *Acroneuria*, *Neoperla* (all Perlidae), or *Isoperla* (Perlodidae) then consult Stark (1989) and Szczytko and Stewart (1979) for methods of extruding and preserving aedeagi. If a bulk sample without regard to sex or preparation is desired, white trays with ethanol may be placed at the base of the sheet to catch adults that invariably lose their balance and fall. One can walk away from this apparatus, set up others elsewhere, and return later to pick up the specimens. Alternatively, a bucket-type trap, with the light held vertically, can be fitted with a funnel above the preservative container.

In situations where air temperatures are not high enough (must be in the low 20 C range, but higher is better), such as in the mountain west, ultraviolet traps are not useful. Other traps can be deployed for extended periods including the Malaise trap, a tent-like device constructed of netting. This trap has many designs, but generally resembles streamside vegetation. It is composed of folded netting that effectively channels crawling and flying adults to an elevated position, where they fall into a preservative reservoir at the top of the trap. Pitfalls are also useful and can be made in the hundreds with little cost. Cans, or other containers, are sunk into the ground along the stream and partially filled with preservative and usually covered to keep out rain. These are very effective for brachypterous and micropterous species. Sticky traps are also used for collecting adults. Pieces of wood, tree trunk, or plastic are coated with a sticky substance (Tanglefoot® is an example) and placed at locations where stonefly emergence is likely. The specimens are removed with a solvent before being preserved. Passive methods are most effective for winged adults of stonefly species that cannot be readily collected with other more active means of capture. Adult specimens can also be collected from locations where incidental or accidental capture has occurred. Stonefly adults have been collected from lighted areas such as gas station pumps, fountains, greenhouses, and large parking lots.

Stonefly Rearing

Stonefly nymphs are reared because many nymphs cannot be identified to the species level without the adult. This lack of association of adults and nymphs of the same species has plagued

researchers since the beginning, and there are still many species unassociated (Stark in McCafferty et al. 1990). Because rearing results in a shed exuvium that retains last-instar nymph characters, it provides a taxonomic association between the nymph and adult stages; this is important because most keys and new species descriptions are based on the adults, and nymphal keys have not yet been developed for some genera. The conditions that are necessary for successful rearing depend on the functional feeding type of the species, season of collection, and the amount of remaining nymphal growth needed before emergence. Most stoneflies require food and moving water to survive for extended periods, but pre-emergent nymphs (those with darkened wingpads) can be reared without these requirements as long as dissolved oxygen remains good. Stonefly nymphs also require a rough surface for emergence because tarsal claws need to be anchored for the winged adult to shed the last instar exuvia. Cups made of foam or rearing chambers made of screen material have both been used with success.

Most winter-emerging species are easy to rear because they are herbivorous (just feed them conditioned, deciduous tree leaves from the stream where they originated) and because dissolved oxygen concentrations are high at low winter temperatures. Place them in eight or 12 ounce Styrofoam cups with a couple centimeters of stream water, covered with a lid, and store them in a chest cooler (more to prevent freezing) in a garage or cold basement. Check them daily to give the insects a daylight cue.

Predaceous stoneflies are often territorial, and if reared for long periods, are usually placed in isolation from other nymphs where they can more easily be fed without being injured by the cage mates. Rearing chambers can be made from the same cups as before and placed in a temperature-controlled environmental chamber or circulating artificial or natural stream with appropriate water temperature. Summer species can be reared in static situations with adequate aeration; in the field, battery operated pumps fitted with air stones work well for transporting and rearing. If recirculating laboratory streams are used, chambers can be placed on floating rafts made of foam, fitted with brackets, or anchored onto concrete blocks or bricks. Predaceous species can be fed small mayfly nymphs or caddisfly larvae, or the larvae of several dipteran families including the Chironomidae, Culicidae, and Tipulidae.

Depending on the rearing situation, emergence can be induced by manipulating water temperatures in the rearing chambers when nymphs have reached the last instar. In the laboratory, *Pteronarcys* nymphs have been artificially induced as much as 2-3 months ahead of their normal emergence period. After successful emergence, allow adults to harden their exoskeleton, then periodically removed to meet whatever objectives have been set. Adults can be stored alive in screen cages or with glass tubes fitted with a moist sponge.

Most stonefly adults and nymphs are stored in preservative fluid now a days. However, if your intent is to use specimens for molecular systematics or conservation genetics, seek the advice of those who do such work. Storage in 70-80% EtOH will not stop the degradation of DNA or RNA; base-pair lengths may be only 400-600 units long after only a few years in EtOH. Preservation in 95% EtOH, at near freezing temperatures is better, but if these specimens are to be stored for any length of time, they should be frozen immediately in an ultra cold freezer. Dr. Michael Whiting of Brigham Young University is building a frozen tissue collection of stoneflies and other aquatic insects and would be a great collaborator for such molecular work, and an appropriate repository for frozen specimens. Similarly, the American Museum in New York has made a large investment into fully curated cold storage of specimens. There are also those who say that pinning of adult stoneflies (and other aquatic insects) should see a resurgence, because dry storage is nearly as good for long-term preservation of DNA as freezing.

A REFERENCE GUIDE TO NORTH AMERICAN PLECOPTERA IDENTIFICATIONS

The following section is designed to be a quick reference guide to the identification of North American stoneflies with a focus on the nymphs. We begin with a quick reference key for identifying major nymphal characters of the families found in North America. It is then followed by sections that provide selected references in the literature and on the web that are commonly used in identification.

The main reference to the identification of stonefly nymphs is Stewart and Stark (2002), which is up to date and includes all currently described genera. A list of keys to stoneflies in states or regions is provided to assist with identifications specific to a given region. Some selected keys to genera and families are also provided. However, references to taxonomic checklists to states or regions are not provided. The USGS website produced by Baumann and Kondratieff summarizes the known distributions of stoneflies in the United States by county (<http://www.npwrc.usgs.gov/resource/distr/insects/sfly/sflyusa.htm>).

A synopsis of the key characteristics of nymphs of North American Plecoptera families

Carnivores- paraglossa much longer than the glossa.

Perlidae- branched gills on the thorax

Perlodidae- cerci as long or longer than abdomen, hind wingpads divergent.

Chloroperlidae- cerci shorter than $\frac{3}{4}$ length of abdomen, hind wingpads parallel.

Detritivores - glossa and paraglossa about the same length so that tips lie in a relatively straight line.

Pteronarcyidae- branched gills on abdominal segments 1 through 2 or 3.

Peltoperlidae- simple gills at base of some legs; thoracic sternal plates shield like, overlapping; roach like appearance.

Taeniopterygidae- hind wing pads divergent; tarsal segments 1 and 2 about the same size (=subequal).

Nemouridae- wing pads divergent; hind legs extend to tip of abdomen; tarsal segment 2 smaller than 1.

Capniidae- hind wing pads parallel, reduced or absent; pleural fold on Ab1-9; tarsal segment 2 smaller than 1.

Leuctridae- hind wing pads parallel, longer than wide; pleural fold on Ab1-7 at most; tarsal segment 2 smaller than 1.

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Web-Based Keys and Checklists

A web-based key to families with photographs.

http://www.unb.ca/fredericton/science/biology/Invertebrate_key/Plecoptera/Plecoptera_key_NB.htm

USGS Checklist of Stoneflies. This contains known records for stonefly distributions at the county level in United States with supporting references.

<http://www.npwrc.usgs.gov/resource/distr/insects/sfly/sflyusa.htm>

Keys to Regional Fauna

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Jewett, S. G. 1956. Plecoptera. pp. 155-181. In Usinger, R. L. (ed.). *Aquatic Insects of California*. University of California Press, Berkeley.

Colorado (mountains):

Ward, J. V., B. C. Kondratieff, and R. Zuellig. 2002. *An illustrated guide to the mountain stream insects of Colorado*, 2nd Edition. University of Colorado Press.

Connecticut:

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Michigan: http://insects.ummz.lsa.umich.edu/~ethanbr/aim/Keys/Plecoptera/id_pom.html

Minnesota:

Harden P. H., and C. E. Mickel. 1952. The stoneflies of Minnesota (Plecoptera). Technical Bulletin of the Minnesota Agricultural Experimental Station 201. 1-84.

North and South Carolina:

Unzicker, J. D. & V. H. McCaskill. 2002. Plecoptera, pp 5.1-5.50 in Brigham AR, Brigham WU & Gnillka A (eds). Aquatic insects and oligochaetes of North and South Carolina. Midwest Aquatic Enterprises, Mahomet, IL.

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Ozark and Ouachita Mountains:

Poulton B. C. and K. W. Stewart. 1991. The stoneflies of the Ozark and Ouachita Mountains (Plecoptera). Memoirs of the American Entomological Society 38. 116 pp.

Pacific Northwest:

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Texas:

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Utah:

Gaufin, A. R., A. V. Nebeker, and J. Sessions. 1966. *The Stoneflies (Plecoptera) of Utah*. University of Utah Press, Salt Lake City. 93 pp.

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Kondratieff, B. C., and J. R. Voshell, Jr. 1982. The Perlodinae of Virginia, USA (Plecoptera: Perlodidae). *Proceedings of the Entomological Society of Washington* 84: 761-774.

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RESEARCH NEEDS IN NORTH AMERICA

Stark (in McCafferty et al. 1990) discussed the status and needs of systematics of North American stoneflies. He predicted that about another 50 species would be described. We have surpassed that total after only 15 years (Fig. 1), so it is conceivable that the total fauna could exceed 700 species within a few decades. California and the Cascade Range were suggested as important sources of new species and new genera in North, this has been born out. A major need was for generic revision. Several have taken place, examples being *Suwallia* (Chloroperlidae) Alexander and Stark (1991), *Isocapnia* (Zenger & Baumann 2004A) and *Paracapnia* (Zenger & Baumann 2004B) both of the Capniidae, and there are others to be published in the near future. These revisionary studies will help standardize comparisons of external and internal characters, building a better knowledge base for phylogenetics. Still, only about 45% of the nymphs of North American species are known, so rearing continues to be important.

It has become readily apparent that stoneflies are being decimated by human agriculture, logging, and urbanization. We should hope that the case study in Illinois is not indicative of what is taking place in other areas. Documenting the stonefly fauna of unique and isolated habitats is still important to do. It is also important to inventory stonefly fauna in areas that have been well collected in the past, lest the community begins to change without one noticing.

There is a great need to pull all of the existing stonefly records from museums, literature sources, and from reliable biomonitoring efforts into a single source. This information would help conservation organizations to prioritize watershed improvement and the linking of key watersheds to reduce fragmentation of habitat. These data could also be used to establish imperilment rankings where enough data exist and tell us where the gaps are. Basic research questions could also derived from such a resource. Knowing the locations of genetically isolated species could lead to several theses on the conservation genetics of stoneflies and better know the effects of fragmentation. This data source would improve with more people providing specimens for voucher. So, please deposit specimens in accredited regional or national museums.

It has been estimated that up to \$1.5 billion dollars a year is spent on stream restoration projects in the United States. It is hoped that these improvements might lead to recolonization by once extirpated stoneflies. One could develop basic research questions centered on the reintroduction of stoneflies into their former range. Currently, a project is under way to reintroduce *Pteronarcys californica* to portions of the Provo River, Utah, where it was lost. Currently, this entails kick netting all pteronarcids (*Pteronarcys* and *Pteronarcella*) from lower reaches of the river and transporting them to upstream sections. In other situations, one might look for pinned museum specimens to molecularly determine the original ecotype (combination of haplotypes) for the area and try to find the same or similar ecotype in regional streams to act as stock for reintroduction. Following changes in the community structure of system that has lost its large predatory stoneflies could be rewarding research project.

Other entomologists have built sophisticated database infrastructures for maintaining vast amounts of systematic and nomenclatural data for their families or orders. The order Plecoptera is small enough that this is feasible to do. The production of on-line catalogues, interactive keys, and nomenclature databases should be a priority. Access to these resources should be available to all comers.

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