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ABSTRACT OF THESIS

BUILDING WEB-BASED INTERACTIVE KEYS TO THE HYMENOPTERAN FAMILIES AND SUPERFAMILIES

Linear keys are the standard for identification in insect taxonomy. However, with the use of the world wide web and non-linear key formats, a variety of key architectures are now available for creating pleasing and informative keys for wide-spread use. This project uses DELTA (Description Language for Taxonomy), created by the CSIRO Division of Entomology, to produce a non-linear key to the Hymenoptera. A variety of tools for illustrating morphological characters were implemented to clarify the key characters and the keys are intergrated into the National Science Foundation's "Assembling the Tree of Life" initiative for the Hymenoptera.

KEYWORDS: Interactive Keys, Hymenoptera Taxonomy, DELTA (Description Language for Taxonomy), Web-Based Education, Tree of Life

> Katja Chantre' Seltmann December, 2004

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BUILDING WEB-BASED INTERACTIVE KEYS TO THE HYMENOPTERAN FAMILIES AND SUPERFAMILIES

By

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THESIS

The Graduate School University of Kentucky

THESIS

A thesis submitted in partial fulfillment of the

at the University of Kentucky

By

Lexington, Kentucky

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Chapter 1: Introduction

Computer Terminology

Throughout this thesis computer related terminology is formatted differently than the body of the text to clarify the function of the term. All file names are in single quotes (i.e. 'specs'), interactive key program names are in all capitals (i.e. DELTA), commands initiated from the program menu bar are underlined (i.e. <u>file>find file</u>), formal names and direct quotes from individuals or from the Hymenoptera Interactive Key are placed in quotations (i.e. "Do the antenna sit on a shelf?"), program windows and fields are in bold lettering (i.e. **Item edit** window), and titles of publications and manuals are italicized (i.e. *Hymenoptera of Costa Rica*). A window is defined as an interface contained within a program for adding information to the program. A field is the actual location on the window in which information is added and a tab is button on a window that links to a new window. Figure 1-1 illustrates a window, the **Character Edit** window, which contains three fields and the five standard tabs.



Figure 1-1: Computer related terminology

Hymenoptera Biodiversity

Hymenoptera is one of the three largest orders of insects. We recognize twenty superfamilies and eighty-nine extant families, with an estimated total of 300,000 species worldwide (Goulet & Huber, 1993). Evidence suggests that the Hymenoptera is the most species-rich group in temperate regions (Gaston, 1991) and there are more species in the parasitoid family Ichneumonidae than the combination of all mammal and bird species (Wilson, 1988; Gaston, 1993). The order is exceptionally diverse: from thumb sized Vespidae that provision their young with larvae of crop pests, wingless Chrysididae egg parasitoids, to dust-sized Mymaridae commonly called "fairy-flies" due to their feather-like wings. Although this diversity is often overlooked, the common names ant, bee, sawfly, and wasp indicate the importance of Hymenoptera in our society (Gauld and Bolton, 1988). Arguably the order is best known for the few social families that contain many individuals; encountered as a result of their ability to sting, bite, and infest houses. They are useful to us as biological control agents, primary pollinators for food production, and forest decomposers.

However a person regards the members of the Hymenoptera, either as pests, assets or curiosities, it is essential to identify them. Identification keys to the order are almost as diverse as the Hymenoptera themselves. A key is defined as "a taxonomic device by which objects are identified based on suites of characters or character states" (Gordh & Headrick, 2001). They exist in multiple formats including pictorial keys, interactive keys and dichotomous keys. Pictorial keys have a habitus image coinciding with a taxonomic name, such as Borror & White's (1970) *Peterson's Field Guide to Insects*. Dichotomous keys are

"a device of logic used in taxonomy to identify taxa. Specifically, dichotomous keys consist of statements about characters or character states which are provided in alternative contrasting statements arranged in couplets. Based on the correctness of the statement with regard to the specimen at hand, the user is directed to other contrasting statements and ultimately the name of the taxon" (Gordh & Headrick, 2001).

Unlike dichotomous keys, interactive keys are non-linear. The user, defined as any individual identifying a specimen using an interactive key, may choose any character from a list of characters. These characters correspond to an exhaustive list of taxa, and taxa are removed from the list as character states are selected. Pictorial keys and dichotomous keys are traditionally found in print format where interactive keys exist only as computer programs. Besides being

more efficient, interactive keys have many advantages over traditional dichotomous keys. They are easy to update, can be distributed over the internet, and may contain full color images at low cost; vastly different from the high cost of printing with color illustrations.

Interactive keys, first termed polyclave keys, were developed around 1969 with Hansen and Rahn's, *Determination of angiosperm families by means of a punched-card system* (Johnson, 1980). These early keys were all hand punched; a laborious process, limiting their production (Johnson, 1980). In 1974, Morse created the first matrix-generated polyclave program called CARDKEY. Polyclaves that use a combination of character state choices began in 1975. This form of polyclave allowed for more character choices and more detailed keys (Pankhurst et al, 1975). INTKEY, the interactive key used to create the Hymenoptera family keys presented in this thesis, was developed in 1993. Version 4.00, for MS-Windows, was released in May 1995. The present version, 5.11, was released in December 2000 (Dallwitz et al., 2000). LUCID, an interactive key similar to INTKEY, was developed by the Cooperative Research Centre for Tropical Pest Management (CTPM) and the Department of Entomology at the University of Queensland in 1997 (<u>Taxacom Listserv Archive for 1997</u>: LucID interactive key software). INTKEY and LUCID are the current standards for interactive keys. I chose INTKEY to produce the Hymenoptera Interactive Key because of its widespread use.

Hymenoptera Family-Level Keys

The majority of available Hymenoptera keys are either specific to a subset of the order or to a particular geographic location. There are only a few dichotomous keys to the families for the entire order Hymenoptera: Brues et al. (1954), Handlirsch (1925), Borror and Delong's *Introduction to the Study of Insects* (2004), and Mason's (1993) key in Goulet and Huber's, *Hymenoptera of the World*. In the valuable dichotomous key in the *Hymenoptera of Costa Rica*, edited by Paul Hanson and Ian Gould (1995), 61 of the recognized 89 families were included in the book due to their presence in the region. Most interactive keys to members of the Hymenoptera deal with taxonomic groups that a particular lab or researcher is working, with one notable exception. *The British Insects: Families of Hymenoptera* created by L. Watson and M.J. Dallwitz (<u>http://delta-intkey.com/britin/hym/</u>, 2003) contains 55 families. Illustrations in the key come from historically valuable volumes of *British Insects: illustrations and descriptions of the genera of insects found in Great Britain and Ireland* (1824-1840), but they lack detail.

Hymenoptera Phylogeny

Printed dichotomous keys are static, reflecting a phylogenetic hypothesis at the time of publication. Interactive keys, being web based, can be updated rapidly, allowing for access to recent information. Unresolved phylogenies often result in keys that may not have characters to elucidate a family, or keys that require a large number of characters to separate exceptional taxa. As these problems are resolved it is important to incorporate them into identification keys without waiting years for the next edition of a book. Within the Hymenoptera, potentially polyphyletic superfamilies include the Evanioidea and Proctotrupoidea, and of the hymenopteran families presently recognized, 20 may not be monophyletic. These include the Agaonidae, Andrenidae, Aphelinidae, Argidae, Aulacidae, Colletidae, Diapriidae, Megaspilidae, Melittidae, Pergidae, Perilampidae, Proctotrupidae, Pteromalidae, Roproniidae, Scelionidae, Tenthredinidae, Tetracampidae, Torymidae, Xyelidae and Tanaostigmatidae (Sharkey, NSF Proposal). Eight of these families belong to the Chalcidoidea, and for this reason it only included in the Hymenoptera Family Key to superfamily level.

Carl von Linne' (1735), in the *Systema Naturae*, established the class *Insecta* and first recognized categorically the division between the stinging bees and the parasitic wasps. He termed the stinging organisms Apis, Bombylius, Vespa and Crabro, and the parasitic wasps were all clumped together in one group, Ichneumon. Today the stinging Hymenoptera are placed in an informal grouping known as the Aculeata and the non-stinging forms into the Parasitica, together these two groups compose the monophyletic group Apocrita. Apocrita are distinguished from the basal lineages of the Hymenoptera, or Symphyta, by the possession of the "wasp-waist" (Mason and Huber, 1993). This narrow constriction between the propodeum and the second abdominal segment allows for great range of motion for the metasoma Symphytan phylogeny at the family level is mostly resolved, through research by Lars Vilhelmsen (2001) and Susanne Schulmeister (2003). The parasitic Orussoidea are supported as the sister-group of the Apocrita (Rasnitsyn, 1988).

A. P. Rasnitsyn and E. Koenigsmann (Koenigsmann 1976, Rasnitsyn 1969, 1980, 1988) conducted the first attempts looking at the entire order with a series of papers. Rasnitsyn (1988) remained the foundation of all subsequent analysis until 1999 when Fredrick Ronquist et al. published a reanalysis of the Rasnitsyn (1988) paper. The reanalysis included the characters that Rasnitsyn used but did not incorporate those published after 1988 (Sharkey, NSF proposal).

Sharkey and Roy (2002) critically reanalyzed and recoded the wing characters from the Ronquist et al (1999) paper and showed how the original phylogenetic hypothesis was dependent on incorrectly coded wing characters. While results supported traditionally well-known relationships in the Parasitica, the monophyly of the Ichneumonidae + Braconidae, Ibaliidae + Figitidae + Cynipidae, Megaspilidae + Ceraphronidae and Chalcidoidea + Mymarommatidae, these works illustrate how little is known about apocritan relationships at the family level (figure 1-2).



Figure 1-2: Hymenoptera phylogeny from Sharkey and Roy, 2002

Among the Aculeate Hymenopteran, Chrysidoidea, Apoidea and Vespoidea, the superfamily relationships are well resolved. The family limits within the Chrysidoidea are supported. The Pompilidae are generally considered to belong to the Vespoidea; however, their placement is not resolved within the superfamily. Morphological indications even suggest that they may belong to the Apoidea (Brothers and Carpenter, 1993). Although the monophyly is fairly well supported, the family-level relationships in the Apoidea are largely unresolved. In one of the most recent phylogenetic studies the Sternotritidae and Melittidae appear in multiple places within the tree (Brothers, 1993) and the family-level relationships among the specoid wasps are debated. Melo (1997) recognized five families of Apoidea: Ampulicidae, Apidae, Crabronidae, Specidae and Heterogynidae. This resolved the superfamily by placing all bees into the family Apoidea. Michener (2000), presently considered the leading authority on bees, splits

the Apoidea into ten families: Sternotritidae, Colletidae, Andrenidae, Halictidae, Melittidae, Megachilidae, Apidae, Sphecidae, Crabronidae and Ampulicidae.

Objective of Hymenoptera Family Keys

The objective of this project is to create an easily updatable interactive key to the hymenopteran families, excluding the families of Chalcidoidea. The key is intended for distribution through the Hymenoptera Assembling the Tree of Life (HymAToL) web site, a National Science Foundation funded initiative to discover the phylogeny of all known life. The goal is to appeal to a wide range of users, stimulating interest in HymAToL and the mega-biodiverse order Hymenoptera.

Chapter II. Methodology

Organization of Hymenoptera Interactive Keys on the Internet

The Hymenoptera Interactive Keys were organized into a total of eleven separate superfamily and family level keys (figure 2-1). The 'Index.html' web page contains links to each of the eleven keys individually, a link to the DELTA program page, and a brief outline of the keys contained within the pages. A novice user who is not able to identify a specimen to superfamily may begin with the Superfamily Key. This key contains all of the one-family superfamilies: Megalyroidea, Trigonalyoidea, Stephanoidea and Mymarommatoidea, as well as the multifamily superfamilies: Evanioidea, Ceraphronoidea, Proctotrupoidea, Platygastroidea, Cynipoidea, Chalcidoidea, Ichneumonoidea, Chrysidoidea, Apoidea and Vespoidea. The Superfamily Key groups all of the superfamilies of the Symphyta, an informal assemblage of the basal hymenopteran lineages, into a single key. Although the symphytan section of the Hymenoptera Interactive Key contains seven separate superfamilies: Xyeloidea, Pamphilioidea, Tenthredinoidea, Cephoidea, Siricoidea, Xiphydrioidea and Orussoidea, they share similar morphological features and are often considered together by taxonomists when investigating the morphology, taxonomy, and phylogeny of the order.





The justification for having multiple keys instead of one single oversized key is that characters are often useful for only specific taxon. Including all such characters in a comprehensive key would force users to wade through a large number of inefficient characters to arrive at identification. If all characters informative only for individual families were included in one key, the key would be hundreds of characters long. For example, an enlarged midtibial spur is diagnostic for Ctenoplectrini (Apidae). When a key user is trying to identify a specimen known to be a member of the superfamily Apoidea, this spur character may result in a quick identification. However, the character "Does the specimen have an enlarged midtibial spur" is not informative for the majority of the Hymenopteran families. A second advantage to separating keys based on superfamilies is that partially informed users can limit their search simply by going to the key closest to the identification. For example, if the user knows that their specimen is a bee, they can proceed immediately to the Apoidea key.

DELTA (DEscription Language for Taxonomy)

Description Language for Taxonomy, or DELTA, was created at the Commonwealth Scientific and Industrial Research Organization (CSIRO) between 1971 and 2000. DELTA is a taxonomic organizational tool, allowing the author to enter characters, character states, and taxa into an easily readable matrix format. The program is designed for Windows 95 or later and also works in Virtual PC for Macintosh.

The process of creating an interactive key using the DELTA taxonomic data format began with entering the characters and taxa in a DELTA matrix. Information about the type of character, reliability of the character, notes concerning the character, illustrations of taxa, character states, and character dependences were entered.

Once data are entered into DELTA, the program CONFOR can translate the information into multiple formats, including: NEXUS, natural language lists of characters, non-linear keys (INTKEY) or traditional dichotomous keys. CONFOR translates the directive files created by the DELTA program. In order for CONFOR to run, the author must first export the directives as text files and then run the "Action Sets" dialogue from the menu contained inside DELTA, which informs CONFOR which translation from the directives is desired. From this dialogue, CONFOR creates specific directive files for use by other programs such as INTKEY. These

directive files are the program's organizational system, where tasks are compartmentalized into separate files with '.ink,' '.bak' or '.ini' extensions. When translating to the INTKEY format, CONFOR creates the 'toint' file and the 'intkey.ink' file which are the directive files for running an interactive key. The three primary output files for DELTA and CONFOR are the 'chars', 'items' and 'specs' files. The 'chars' file is composed of the list of characters found in the DELTA matrix, the 'items' file lists the taxa, and the 'specs' file dictates how the 'chars' and 'items' files are arranged in the matrix (Dallwitz et al., 1995 onward).

The directive files were manipulated using the **Attribute Editor (Trees)** window (figure 2-3); however, it was often helpful to manipulate the directive files directly with Notepad[©] for Windows operating systems. Manipulated directive files were imported as text files and then saved as non-extension files, replacing the original directive files. If an original directive file and a text file are both present in the directive folder, the program will read the original, not the updated file. For example, if the 'specs' file is saved as a 'specs.txt' file, CONFOR will read the directive file listed simply as 'specs' without the '.txt' extension.

Using DELTA to Create INTKEY

Organization of the sixty plus directive files was addressed before using DELTA. All of the directive files were placed in a single folder. This folder was labeled "directive," and existed inside a larger folder labeled for the title of the specific interactive key. A separate file for the images used in the key was created inside the directives file. This became increasingly important as the key expanded and the number of image files increased. The program requires that all image files be contained in the same folder. The initiation file for the DELTA program, or '.dlt' file, was placed outside of the directives folder, which proved useful in locating the file. Additional directive files that were manipulated using Notepad© were backed up in a separate folder outside of the directives file. When DELTA translates "Action Sets" using CONFOR, specific directives may be changed back to the program's default setting. These directives include the 'contents.ind' file for manipulating references and acknowledgments, and the 'toolbar.inp' file which indicates button function.

INTKEY is one of the possible translations of the DELTA matrix using CONFOR. Recognizing the purpose of the original matrix is helpful in making decisions when entering data into the matrix. Brackets are used around the character that will be replaced in the final

description by the character state (Dallwitz et.al, 1993). For example, a character that is entered as "integument <color>" with character states "blue or blue absent" will be translated into the final description as "integument blue" as the question of color is replaced by the state blue (figure 2-2). However, a character written in the manner "integument <color>" is not a typical way an individual encounters a question. Generally, when a question is addressed it is in the form of a complete sentence such as, "Is the integument blue?" with character state choice as "yes or no." Character state descriptions resulting from a character descriptions. The description resulting from the sentence "Is the integument blue?" with state choice "yes" results in the confusing character state description "Is the integument blue?" with state choice "yes" results in the

Figure 2-2: Difference in final descriptions depending on the style of writing initial characters

Format of Character	Character State	Resulting Description
integument <color></color>	Blue	integument blue
Is the integument blue?	Yes	Is the integument blue?yes

The Hymenopteran Interactive Keys used complete sentences to enter the characters. Although final descriptions may be jumbled these were not the objective of the present effort. If the original intention of the project was to create taxon descriptions, the information would be entered in DELTA using brackets.

Upon first opening the DELTA program, the author is directed to the **Attribute Editor** (**Trees**) window in DELTA (figure 2-3), and may use this window as one of two windows to code and view all of the characters and taxa in the program. The other window is the **Attribute Editor** (**Grid**) window (figure 2-4). These windows may be interchanged as desired from the menu <u>View>Attribute Editor (Grid</u>), or <u>View>Attribute Editor (Trees)</u>, to enter data.

Figure 2-3: Attribute Editor (Trees) window

🛕 Proctotrupoidea family key.dlt - Attribute	Edit (Trees)	_ 🗆 🔀
Pelecinidae Vanhorniidae Monomachidae Austroniidae Diapriidae Peradeniidae Proctotrupidae Roproniidae Peloridae Portotorenyxidae Naamingidae Taxon field	 U 5. Is apparent metasomal tergum 1 the largest? U 6. Is tergum 2 (true or apparent) several times longer than tergum 3 (true U 7. Is the metasomal segment 1 (true or apparent) at least 3 times longer than U 8. Is metasomal segment 1 is at least three times longer than wide 2. metasoma segment 1 is at most twice as long as wide U 9. Is there a rectangular lobe at the base of the tarsal claws? U 10. In which biogeographic realm was the specimen collected? U 12. Is the scape at least 2.5 times longer than wide? U 13. Are tubular veins present on the forewing? U 14. Is the stigma of the forewing clearly indicated? U 15. How many flagellomeres does the antenna have (not including the an U 16. Does the forewing have a first discal cell? U 17. What is the shape of the first discal cell of the forewing? U 18. Is vein (Rs+M)a of the forewing tubular? 	or apparent) n wide? Illy? field ellus)?
<u>B</u> <u>I</u> <u>U</u> x ² x ₂ <u>K</u>) C ▼ 1	☐ 1. metasomal segment 1 is ☐ 2. metasoma segment 1 is	at least thr at most twic

Figure 2-4: Attribute Editor (Grid) window

🋕 Proctotrupoidea family key.dlt - Attribute Edit (Grid)											
	9	10	11	12	13	14	15	16	17	18	19 🔺
	Is there a	In which	Do the	Is the	Are	ls the	How	Does the	What is	ls vein	ls
	rectangula	biogeogra	antenna	scape at	tubular	stigma of	many	forewing	the shape	(Rs+M)a	metas
	lobe at	realm	appear to	least 2.5	veins	the	flagellome	have a	of the first	of the	segmi
	the base	was the	sit on an	times	present	forewing	does the	first	discal cell	forewing	one (t
	of the	specimen	antennal	longer	on the	clearly	antenna	discal	of the	tubular?	or
	tarsal	collected?	shelf	than	forewing?	indicate	have (not	cell?	forewing?		appar
2 Vanhorniidae	1	3/5/6	2	U	1	1	11	1	1	1	1
3 Monomachidae	2	1/4	2	1/2	1	1/2	12	2	-	1	1/2
4 Austroniidae	1	1	2	2	1	1	12	2	-	1	2
5 Diapriidae	2	1/2/3/4/	1/2	1	1/2	2	2-13	2	-	1/2	2
6 Peradeniidae	2	1	2	2	1	1	11	2	-	2	1
7 Proctotrupidae	1/2	1/2/3/4/	2	2	1	1	11	2	-	2	2
8 Roproniidae	U	3/5/6	2	2	1	1	12	1	1	1	1/2
9 Heloridae	U	1/2/3/4/	2	2	1	1	11	1	2	1	2
10 Proctorenyxidae	U	3/6	2	2	1	1	12	1	1	1	2
11 Maamingidao	2	1	1	1	1	2	12-13	2	_	1	2
1 I. yes, greater than 2 cm 2. no, less than 2 cm											

Creating a matrix was the natural first step in developing an INTKEY. Taxa and habitus images were entered in the **Item Edit** window (figure 2-5). This window is accessed from the menu, <u>View>Taxon editor</u>, or by double-clicking in the **Taxon** field from the **Attribute Editor** (**Trees**) window. Each taxon has an associated number, which corresponds to its location on the character tree or in the matrix.

Entered in the **Subject text** field (figure 2-5) were the descriptive names for the images used in the final interactive key. Information concerning specific taxa was posted in the **Developers notes** field concerning needed improvements, corrections, and observations. Once the taxa had been entered, the **Done** button, in the bottom left hand corner, was clicked to close the window.

ixon Num <u>b</u> er:	Edit taxon name	Edit taxon name:			
1Sejer	Pelecinidae				
iges mage files: pelicinidae.jpg MALEPELECINIDAE.jpg			Subject text:		
		Settings			
		Dis <u>p</u> lay			
		Dis <u>p</u> lay <u>A</u> dd	Image gound		

Figure 2-5: Item Edit window in DELTA

Characters were entered in a similar fashion. The **Character** field in the **Attribute Editor (Trees)** window is double clicked to open the **Character Edit** window (figure 2-6) or it may be accessed from the menu <u>View> Taxon editor</u>.

Character Number:	Edit feature description:	
11 Self Character Type: Unordered multistate Unordered multistate Excluse	Do the antenna appear to sit on an antennal shelf when viewed laterally?	Language: (Not yet implementec Variant: (Not yet implementec
States Notes Images Controls Defined states: 1. yes 2. no	Edit state description:	
Done	$\begin{array}{c c c c c c c c c c c c c c c c c c c $	Help!

Figure 2-6: Character Edit window in DELTA

Characters and Coding the Matrix

DELTA supplies multiple coding options for character types including: unordered multistate, real numeric, integer numeric, ordered multistate, and text. The Hymenoptera Interactive Key used only unordered multistate characters and integer numeric characters. Unordered multistate characters are lists of several discrete choices from which the key user chooses the one most appropriate for their unknown specimen. An example of an unordered multistate character is, "Do the antenna appear to sit on an antennal shelf when viewed laterally?" with character states "yes/no" (figure 3-6). Integer characters are counting numbers and are coded for the entire range for the family. The user enters the number found on their specific specimen. An example of an integer character is the number of antennal segments, or flagellomeres, found on the antenna (figure 3-3).

The abilities of the eventual users of a key were considered when defining character states. Each character state of an unordered multistate must be clear and distinctly separated from other states. Taxa that contain a subtle version of the character state, or that contain both states within the family, were coded "yes/no" for that character.

Translating Characters from a Dichotomous Key to an Interactive Key

The Hymenoptera Interactive Keys were partly based on the 1993 dichotomous keys in Hymenoptera of the World: an Identification Guide to Families, edited by Henri Goulet and John Huber. Translating a dichotomous key into an interactive key creates challenges for coding. Often dichotomous keys will rank multiple characters from the most informative to the least, include characters that are only present in a few groups within a family, and include characters that are relevant only for a few terminal taxa. Interactive keys allow the user to approach the characters from any direction in the key, so each character must be self-contained and not dependant on answering a prior character correctly. Also, dichotomous key characters beginning with statements such as "many" or "a few" are not useful or necessary in interactive keys. Unordered character states in an interactive key require that taxa with "a few" to be coded for both presence and absence of that character, rendering them uninformative. For example, in the Ichneumonoidea interactive key the vein 2m-cu is very useful for separating Ichneumonidae from Braconidae, as the majority of ichneumonids have this vein and braconids generally do not. One rare Braconidae, Apozyx penai, does have vein 2m-cu, so if the vein is present, you either have an ichneumonid or a specimen of Apozyx penai. If this character were to be coded in the interactive key matrix both states (present/absent) would be required for the Braconidae (figure 2-7).

	Is vein 2m-cu present?
Ichneumonidae	Present
Braconidae	Present or absent

Figure 2-7: Character matrix with Apozyx penai and most Braconidae combined

To overcome this apparent disadvantage, *Apozyx penai* was separated in the taxon list, leaving "Most Braconidae" for all of the braconids that do not contain vein 2m-cu. A second character, "Cyclostome mouth?," separates *Apozyx penai* easily from the Ichneumonidae. The resulting matrix (figure 2-8) increases the effectiveness of this easily observed character, the 2m-cu vein, because "Most Braconidae" may now be coded as "Absent" and all Ichneumonidae "Present," distinctly separating the two taxa.

	Is vein 2m-cu present?	Cyclostome mouth?
Ichneumonidae	Present	Absent
Apozyx penai	Present	Present
Braconidae		
most Braconidae	Absent	Present or absent

Figure 2-8: Character matrix with *Apozyx penai* and most Braconidae separated.

The keys are not intended to key every specimen of Hymenoptera in the world. If every rare and exceptional species were included the keys would be long and cumbersome. The qualifying condition of being common enough to separate out a taxon was somewhat arbitrary. Generally, a taxon was considered common enough to be separated if a collector was likely to find a specimen with a "moderate" amount of collecting. A taxon was not considered common enough if it is "rarely" collected. These decisions were made with the aid of collaborators who have extensive collecting experience within their area of taxonomic expertise. Taxa that were exceedingly rare were only mentioned in the **Notes** section of the key and illustrated there with a photograph.

Characters are ordered in INTKEY based on those characters with states that tend to separate the greatest percent of the remaining taxa. Those that possess the ability to differentiate 50% of the remaining taxa are put at the top of the character list. Often Hymenoptera with reduced wings have unusual morphological features, but these characters are usually only important in separating these taxa from others with reduced wings. When all taxa are being considered these characters are near the bottom of an exhaustive list of characters; however, after selecting the "wings reduced or absent" state of the character "Are the wings fully developed?" all characters associated with fully developed wings are removed and those characters that are most efficient at separating taxa with wings reduced or absent move to the top of the list. Also, in the **Remaining Taxa** field (figure 3-2), only those taxa with reduced or absent wings will be displayed.

Many characters in a matrix were included simply to distinguish one taxon. These were generally not informative, or even not applicable, to other taxa. In such cases these cells were coded with a U, which can also mean unknown. For example, wing characters are not applicable

to taxa with reduced wings. In INTKEY, when a wing venation character is used, this character does not affect taxa with a U coded for the character.

The intended audience for the Hymenopteran Interactive Key is wide: high school teachers, undergraduates, amateur entomologists, graduate students in insect taxonomy, and professional taxonomists researching Hymenoptera. In order to increase the potential for diverse interests groups to use the key, terminology was standardized across families; characters were worded in complete sentences and the use of technical language was minimized. Wing terminology, reinvented by many authors, was standardized from Sharkey and Wharton (1997). The interpretation of technical language may be a great obstacle for a novice key user (Jarvie & Stevens, 1998). A term such as "torulus" was changed to the vernacular "antennal insertion" and "seta" was changed to "hair." Also, each of the keys was linked to a glossary of Hymenoptera terminology.

Collaborator Involvement

The initial matrix mimicked the characters in the dichotomous keys found in *Hymenoptera of the World: an Identification Guide to Families.* The matrix was coded by extrapolating from the dichotomous key which was then sent to collaborators specializing in particular families. Specialists were asked to review the characters and character state codings, code the parts of the matrix that could not be extrapolated from the dichotomous key, and send specimens for photography. The list of collaborators includes: Matthew Buffington at the University of California Riverside (Cynipoidea), Jim Carpenter at the American Museum of Natural History (Vespoidea), Andrew Deans at the University of Illinois (Evanioidea), Lynn Kimsey at University of California Davis (Chrysidoidea), Lubomir Masner at the Canadian National Collection of Insects (Proctotrupoidea and Platygastroidea), Michael Ohl at Museum fur Naturkunde der Humbolt-Universitat (Apoidea), Laurence Packer at York University (Apoidea), Jean-Yves Rasplus at Campus International de Baillarguet (Chalcidoidea), Susanne Schulmeister at the American Museum of Natural History (Symphyta), David Smith at the Smithsonian Institution (Symphyta), Michael Sharkey at the University of Kentucky (Ichneumonoidea), Lars Vilhelmsen at the

(Symphyta), Matthew Yoder at Texas A&M University (Proctotrupoidea) and David Wahl at the American Entomological Institute (Ichneumonoidea). Depending upon the preferences of the

collaborators the files were sent as a Nexus file, DELTA file, Macrosoft Excel[©] file, Nexus Data Editor file, Winclada file, or paper copy.

Nexus files were exported directly from the DELTA editor and were imported directly into either PAUP, Nexus Data Editor or Winclada. The 'chars', 'items' and 'specs' directive files were translated into Excel© files using Taxisoft©, a data conversion program written by E.J. Gouda. This is a freeware program available on the internet at the Free Delta website (http://www.websamba.com/freedelta). The three DELTA files were placed in the same folder as the 2exl.bat execution file. The "*DATA BUFFER SIZE 4000" line in the 'specs' file (figure 2-9) was deleted and the file resaved so the program could run correctly.

Figure 2-9: 'specs' directive file illustrating input into Taxisoft©

*SHOW ~ Dataset specifications. *DATA BUFFER SIZE 4000 *NUMBER OF CHARACTERS 25 *MAXIMUM NUMBER OF STATES 6 *MAXIMUM NUMBER OF ITEMS 11 *CHARACTER TYPES 15,IN *NUMBERS OF STATES 10,6 *IMPLICIT VALUES *DEPENDENT CHARACTERS 16,2:17 *MANDATORY CHARACTERS

The translation into tab-delimitated text occurred when the '2exl.bat' execution file was double clicked and the 'itemtab.txt' file was opened in Excel[®]. When opening the 'itemtab.txt' the option "read the '.txt' characters only as text" was preferred, because otherwise some of the characters contained within the 'items' file may have been read as calendar dates.

Adding Notes and Text to DELTA

Supportive information was added to INTKEY from DELTA. From the **Character Editor** window, notes were entered in the field that comes up after selecting the **Notes** tab. Text was typed directly into the field or pasted from a text editing program such as Notepad[©] or
Microsoft Word[©]. Terms defined within the **Edit character notes** field were underlined using the text edit button. After the **Notes** field was complete, the **Done** button closed the window.

Character Number: 4 Seject Character Type: Unordered multistate Mandatory Exclusive States Notes Images Controls Controlled Edit character notes In some Ceraphronidae the mesoscutt	Edit feature description: How many longitudinal furrows are present on the mesoscutum ? I by um is reduced to a narrow sclerite.	Language: (Not yet implemented Variant: (Not yet implemented Variant)
Done	$\boxed{\begin{array}{c c c c c c c c c c c c c c c c c c c$	Help!

Figure 2-10: Edit character notes tab from Character Edit window in DELTA

The **Edit character notes** field in DELTA (figure 2-10) was particularly useful for explaining exceptional character states or unusual terminology because the data entered in this field was translated into an INTKEY **Notes** buttons. These buttons appear in INTKEY on the same window as the character state illustrations (figure 3-3).

Image Editor and Overlays

Prepared character images were inserted into DELTA from the **Image** tab on the **Character Edit** window (figure 2-11). The same actions were used for importing a taxon image into DELTA, except that the taxon image was entered in the **Item Edit** window, not the **Character Edit** window.

🛕 Ceraphronoidea family 02-26.d	lt [2] - Character Edit	
Character Number: 4 Seject Character Type: Unordered multistate Mandatory Exclusive States Notes Images Controls Co	Edit feature description: How many longitudinal furrows are present o Image: state of the state	n the mesoscutum ?
Image files: FURROWS.jpg	Subject text: Settings Developer's Display Add [empty]	ngtes: id
Done	B I U x ² X ₂ K)	Help!

Figure 2-11: Image files tab from Character Edit window in DELTA

Images imported into DELTA are in 'JPG', 'BMP' or 'GIF' formats. The buttons to the right of the **Image files** field controls the files within the window. Multiple images may be added to the DELTA file. The **Display** button displays the highlighted image, the **Delete** button deletes the highlighted image and the **Add** button adds an additional image. In the **Subject Text** field descriptions were written as they appear in INTKEY. This allows the file name to be listed in a descriptive fashion inside the interactive key. The **Settings** button opens the **Image Settings** window (figure 2-12), which changes the default settings for the images. This panel may also be opened in the **Item Edit** window by choosing <u>View> Image settings</u> from the menu or by right-clicking on the image file. It includes the image font settings, the image saving path, the image overly settings, and the hotspots.

🛕 Ceraphronoidea family 02-26.dlt [3]	- Image Settin	gs 🗕 🗆 🔀
_ <u>D</u> efaults for new overlays	Image Path -	
Center in box	images;din	ectives\images Browse
Include comments	Overlay font	settings
☐ <u>O</u> mit description	<u>D</u> efault:	Times New Roman
, └── Use integral height	<u>F</u> eature:	Times New Roman
Hotspots pop <u>up</u>	<u>B</u> utton:	Times New Roman
Custom popup color		DEFAULT FONT: modeo the default
Choose color	<u>S</u> ample:	for all text boxes Save sample as comment
Button alignment		
align horizontally		OK Cancel Apply

Figure 2-12: Image Settings window in DELTA

Under the **Overlay font settings** field the default font was set as Times New Roman for all text boxes in INTKEY. Changing this setting modifies the image settings throughout the DELTA file. This is also true for the **Image Path** field. The **Image Path** informs the program of where the image folder is located within the directive file. If this setting is incorrect, the **Image Path** will be broken and images will not appear within INTKEY. Changing the default settings does not affect previously entered text. The **OK** or **Apply** button must be pressed for any changes to be applied.

The default overlay used was a clear rectangle which turns black when selected. The function of the overlays for character images is to explain the character and character states, add hotspots for the user to choose which image describes the correct character state, and supply a section for notes and additional comments.

Figure 2-13: Overlays in DELTA



The types of overlays supplied by DELTA are dictated by the type of character. If the image is associated with an unordered multi-state character, as pictured above, two hotspots, **OK** button, **Cancel** button, **Notes** button and character text are provided. If the character is an integer, DELTA does not give hotspots as an option. Instead a box for entering a number is presented. The **OK** and **Cancel** buttons are supplied for an image and the **Notes** button is supplied only if information is entered in the **Notes** field of the **Character Edit** window. All of the buttons were arranged manually, by moving them around with the mouse. The character text was resized to fit the individual images and hotspots were dragged and resized over the entire character state image (figure 2-13). The hotspot area is the part of the image an INTKEY user may click to making a character state choice.

When multiple images are present, an INTKEY user may scroll through the images from the menu, control>next image. Only one image is displayed at a time, so the user was notified of a second image using an instructional text box on the first image. This was done with a **Text** overlay and a statement informing the user that a second image is present. The **Item Edit** window has the same image importation and overlay options as the **Character Edit** window.

Controlling-Attributes and Dependencies

Controlling characters were defined using the **Controlling-Attribute** window. This window is found on the **Controls** tab of the **Character Edit** window (figure 2-14).

🛕 superfamiliesjuly.DLT [2] - Character Edit		
Character Number: Character Number: Character Type: Unordered multistate Mandatory Edit feature description Select Are the wings further Mandatory Exclusive	ption: ully developed (forewing extending past the first um)?	Language: (Not yet implemented Variant: (Not yet implemented Variant)
States Notes Images Controls Controlled by Controlling attribute Are the wings fully developed (forewing extending past the ▼ Defined by states: Regefine 1. yes 2. no, the wings are short (brachypterous) or abs	Makes inapplicable: 8. How many veins run longitudir 10. Is a stigma present on the fo 11. Is vein 2r-m present on the fo 12. Is vein Cu of the forewing defi 44. Does a vein run along the for 48. Is the forewing spoon-shaped 49. Does the hind wing have a st K m	here a conspicuous do he distance between th he ovipositor sheath sh he posterior margin of v long is the pronotum, he forefemur as wide a es the tegula touch a p erqum one (true or app ♥
Done B I U	x ² X ₂ K)	Help!

Figure 2-14: Controlling attribute tab from Character Edit window in DELTA

Controlling characters function to render associated characters inapplicable, if they are not useful after the specific character state has been chosen. This assists the INTKEY user by removing inapplicable characters that are non-informative for the user's specimen. For example, "wings absent" was defined as a controlling attribute for characters that deal with wing venation. Therefore if an INTKEY user were to choose the character state "wings absent," the wing venation characters would be removed from further consideration in the key.

To create a controlling-attribute, the intended controlling character's **Character Edit** window is opened. In the above example, "wings absent" was defined as the controlling state of the character "Are the wings fully developed," so the **Controls** tab was opened from the **Character Edit** window for this character. The character states for the character are listed in the **Defined by states** field. When the intended character is double-clicked it turns into bold type and appears in the **Controlling attributes** field. After a controlling attribute is defined the characters it makes inapplicable are defined. The list of characters on the far right of the window consists of the entire list of characters contained within the key. A chosen character is highlighted and the south of the character will then move into the **Makes inapplicable** field. It is possible to have both character states defined as controlling attributes.

Once a character state is defined as a controlling attribute it may be used to control other characters. This is done in the **Controlled by** window (figure 2-15).



Figure 2-15: Controlled by tab from Character Edit window in DELTA

Controlling attributes for characters are entered from the **Controlled by** tab under the character intended to be affected. A character listed in the **Defined controlling attribute** field is clicked followed by the ______ button; moving the character to the **Made inapplicable by** field. Controlled characters are seen in both the **Attribute Editor** (**Grid**) and the **Attribute Editor**

(**Tree**) windows as red lines and grayed out boxes. The Edit, Variant, and Language options found on the **Character Edit** window are not functional at the present time.

Character dependencies allow for flexibility within the key by creating characters specifically designed for grouping other characters. For example, in the Apoidea key, the character "Is your specimen a male or a female," is obviously not a keying character as states "male and female" were coded for every taxon. However, many of the characters in the Apoidea key are sex specific and the use of character dependencies for this character allows the INTKEY user to remove a large number of characters that do not pertain to the sex of the specimen being identified.

DELTA files that contain controlling attributes are not exported successfully into other formats, including NEXUS files. If a DELTA file was intended for use in an interactive key and as a NEXUS file, it was saved as two files; one with dependencies and controlling attributes for INTKEY; and one without for NEXUS.

Exporting DELTA Directives to INTKEY

Directives are exported before CONFOR can be used to create an INTKEY. Directives are exported from the menu, <u>File>Export Directives</u> (figure 2-16).

Select DELTA files to export	? 🛛
Export directory: C:\Katja\charater lists\sup	erfamily\directive Change
SPECS" file	Do not export
CHARS" file	
Other directives files cimages (C) cnotes (C) dist (D) headc (C) intkey.ink (I) key5 (K) key5a (K) layout (C) mark.htm (C) mark.ttf (C) ofiles (C)	type for ey
OK Car	cel Help

Figure 2-16: Select DELTA files to export window

The **Select DELTA files to export** window allows the author to choose which directives to export and where to export them. The **Change** button allows you to change the export location directives will be exported. It was useful to not export directives (i.e., 'contents.ind,' 'toolbar.inp') that were manually manipulated in Notepad[©] because the DELTA program default would overwrite the directives contained within the directive folder. After directives were exported, CONFOR was used to create INTKEY by operating from the **Actions** window (figure 2-17). The **Actions** window was accessed from the menu <u>View>Action Sets</u>, then the option "translate into INTKEY format" was highlighted in the **Confor** field and finally the **Run** button was clicked. The product was the 'intkey.ink' files that are the initialization files for INTKEY and are the files that a key user must access in order to run the program.

ction set: TRANSLATE INTO INTKEY FORMAT				<u>R</u> un
Action	File name	Imported/exported		_
[Not specified]	tlinks	9/29/2004 2:08 PM		Done
[Not specified]	tlinks.txt	9/29/2004 2:08 PM		
Print the character list - HTML.	printch	9/29/2004 2:08 PM		
Print the character list - RTF	printer	9/29/2004 2:08 PM		
Translate into DIST format. 📂	todis	9/29/2004 2:08 PM		Edit
Translate into INTKEY format.	toint	9/29/2004 2:08 PM		
Translate into KEY format.	tokey	9/29/2004 2:08 PM		
Translate into natural language - HTML	tonath	9/29/2004 2:08 PM		
Translate into natural language - RTF, separate file for each tax	tonatsr	9/29/2004 2:08 PM	<u> </u>	<u>)</u> elete
Translate into natural language - RTF, single file for all taxa.	tonatr	9/29/2004 2:08 PM		
Translate into Nexus format.	tonex	9/29/2004 2:08 PM		
~ Character headings (for printing the character list).	headc	9/29/2004 2:08 PM		
~ Character images.	cimages	9/29/2004 2:08 PM		
~ Character list.	chars	9/29/2004 2:08 PM		
~ Character notes.	cnotes	9/29/2004 2:08 PM		
~ Dataset specifications.	specs	9/29/2004 2:08 PM	~	
<				

Figure 2-17: Actions window in DELTA

Creating Buttons: Changing the 'toolbar.inp' and 'Contents.ind' Directives

Buttons were created to link the Hymenoptera Family Keys to a web-based glossary of terms found within the key and to an introduction to the key and a list of references. The button images were created in Adobe Photoshop[®] at the size of 50 pixels wide by 52 pixels long, which is the maximum button size. If a button image is larger than 50X52 it will not appear in the toolbar of the key. Button images were saved as '.jpeg' files in the directives folder. Buttons were defined in the 'toolbar.inp' directive file (figure 2- 18) by using Notepad[®] to manually add the new information tags. The text document 'Intros.jpg' was defined as the introduction button and 'glossary.jpg' was defined as the glossary button. The command "FILE DISPLAY" was modified in the 'toolbar.inp' directive file linking the Hymenoptera glossary with the 'glossary.jpg' button, causing the Hymenoptera glossary to open when the INTKEY user clicks the glossary button. The "CONTENTS contents.ind" command was written in the 'toolbar.inp' directive file to direct the 'Intros.jpg' button to open another directive file, the 'contents.ind' file (figure 2-19).

Figure 2-18: 'toolbar.inp' directive file in DELTA

*COMMENT ~ Intkey toolbar.

*DEFINE BUTTON CLEAR

*DEFINE BUTTON intros.jpg

"CONTENTS contents.ind"

"Introduction and references"

"This button allows you to display an introduction to the package, or a list

of references."

*DEFINE BUTTON SPACE

*DEFINE BUTTON glossary.jpg "FILE DISPLAY http://www.uky.edu/~mjshar0/glossary/welcome.html" "glossary" "This button links you with the Hymenoptera glossary to help with identification of entomological terms contained in the key"

The 'contents.ind' file was modified to link the words "Introduction," and "References" to the files 'intro.rtf' and 'references.rtf' respectfully. These files are Microsoft Word[©] rich text format files and were saved in the directives folder.

Figure 2-19: 'Contents.ind' directive file in DELTA

Introduction *FILE DISPLAY intro.rtf References *FILE DISPLAY references.rtf

The 'intro.rtf' files were written as introductory documents for each of the Hymenoptera Interactive Keys, listing collaborators, special instructions, and acknowledgements. The 'references.rtf' files were customized depending on the key, listing references that pertain to that particular key.

Linking to Other Interactive Keys: Changing the 'tlinks' Directive

A 'tlinks' directive file (figure 2-20) links one key to another key.

*TAXON LINKS	
# most Cynipoidea/	
http://www.uky.edu/~mjshar0/datasets/cynip.ink <@subject Interactive Key to the	
Cynipoidea Families>	

The 'tlinks' file was modified to create a **Taxon Information** window in INTKEY, which appears after double clicking on a taxon name (figure 3-5). Web link options including species pages, databases, and other keys were linked from the **Taxon Information** window. In the superfamily key, taxon names link the superfamily key to the individual family level keys. For example, the taxon "most Cynipoidea" was linked in the superfamily key to the Cynipoidea INTKEY (figure 2-20). The command "<@subject Interactive Key to the Cynipoidea Families>" appears as "Interactive Key to the Cynipoidea Families" in the **Taxon Information** window, and "http://www.uky.edu/~mjshar0/datasets/cynip.ink" links the text to the 'cynip.ink' file, which is the Cynipoidea interactive key.

Pop-up Introductory Image: the 'toint' Directive

A representative image introducing each key was created to open before the user is allowed to view the interactive key. The image contains a pictorial example of a taxon contained within the key, and author acknowledgements. These images were edited in Adobe Photoshop[©] and saved in the images file as 'title.jpg'. The pop-ups were linked to the interactive key either with the **Action-Sets** window or with the 'toint' file. Below is a portion of a 'toint' file (figure 2-21).

Figure 2-21: 'toint' directive file in DELTA

*INPUT FILE cnotes

*INPUT FILE ofonts

*INPUT FILE cimages

*INPUT FILE timages

*STARTUP IMAGES

title.jpg

*COMMENT If you want Intkey to link to descriptions generated by 'tonatsr', activate the following directive. Before running this file (toint), you must modify 'ofiles', and make additions to your data as described in 'ofiles'.

*COMMENT : INPUT FILE ofiles

The command "STARTUP IMAGES 'title.jpg'" was added to the 'toint file' with Notepad© and imported back into DELTA using the menu, <u>file> import directives</u>. The **Import File** window opens and functions similarly to the **Export File** window. Only the 'tokey' directive file was imported to incorporate the pop-up into INTKEY. A second way to change the 'toint' file is in the **Action-Sets** window. From the menu, <u>View>Action Sets</u> opens the **Action-Sets** window, "translate into INTKEY format is highlighted" and the **Edit** button is clicked. The line *STARTUP IMAGES 'title.jpg' is added to the file and the window is closed. The DELTA file must then be saved for the changes to be incorporated.

Interactive Keys on the Internet: the Intkey.ink File

The 'intkey.ink' file (figure 2-22) was written to point the browser to the location where the information for the key is stored on the web and specifies the image path for the images associated with the key. It opens INTKEY from the html link on the 'welcome.index' web page for the Hymenoptera Interactive Keys. All of the directives, except the 'intkey.ink' file, are kept in a zip file and the images are contained in a folder with the same name.

Figure 2-22: 'Intkey.ink' directive file in DELTA

InkFile=http://www.uky.edu/~mjshar0/datasets/cynip.ink ; Name of this file InitializationFile=intkey.ink ; Name of the Intkey initialization file within the compressed data file

DataFile=http://www.uky.edu/~mjshar0/datasets/cynip.zip ; Name of the compressed data set ImagePath=http://www.uky.edu/~mjshar0/datasets/cynip ; Image path

These 'intkey.ink' files were named after the key with which they are associated. For example, the key to the Cynipoidea 'intkey.ink' file was renamed the 'cynip.ink', thus distinguishing it from the Evanioidea 'intkey.ink' file, 'Evan.ink'. This renaming allowed multiple 'intkey.ink' files to exist in the same folder on the web. All of the files related to the interactive keys are found on the web in one folder, the datasets folder (ftp://www.uky.edu/www/datasets/).

Photography: Creating Images for INTKEY

The quality of photographs, illustrating the taxa, characters and character states, was an important consideration before taking the approximately 600 photographs needed for the Interactive Key to the Hymenopteran Families. Every preparation, from cleaning the specimen to incorporating the image into the key, took approximately three hours. Efforts were made to increase efficiency and streamline the photographic process.

Specimen Preparation

Fresh specimens were used whenever possible to lessen contamination from particulates that collect on older specimens. However, when fresh specimens were not available, older specimens were initially cleaned using an insect pin briefly rubbed on fabric. Dust, moth scales and paper fibers which were attached to the insect jump to the statically charged pin tip as it nears the specimen. If an unwanted particle was difficult to remove, a small amount of insect shellac was placed on the pin tip and was touched to the particle. Specimens were relaxed using a technique developed by Carlos Sarmiento-Monroy (personal communication), in which the

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specimen is exposed to warm, moist air until the specimen is soft enough to move into a desired position. Larger specimens required more time than smaller ones. Dissections were slide mounted using glycerol and a cover slip just prior to photography. Mouthpart and genitalic dissections were cleared using 10% KOH for one hour (Packer, 2003) and were stored in glycerol. Wings were remounted on white label paper and repined with the original specimen.

Lighting Techniques and Auto-montage

The majority of the interactive key photographs were taken with a JVC[®] digital camera, model number KY-F75U, attached to a MJZ 16 Leica[®] microscope. The camera was connected with a firewire cable to an IBM Pentium 4 Intellistation MPro PC running WindowsXP. Software and hardware developed by Syncroscopy[®] were used to generate auto-montaged images. Intact specimens were placed on a block of neutral grey modeling clay on Kodak[®] neutral grey card in preparation for photography. The 14% neutral grey is useful in maintaining the color of the specimen and reducing the amount of reflectivity of the surface (Stephen McJonathan, personal communication) and the modeling clay allows for 180 degree manipulation of the specimen. Fresh specimens were pinned on neutral grey archival paper points. Lights were adjusted differently depending upon the area of the specimen which needed to be photographed. Generally, light was produced from two fiber optic cables positioned on either side, and slightly above, the specimen with a series of Styrofoam[®] cups between the light source and the specimen (figure 2-23). The cups diffused light and reduced glare. One cup was placed directly around the specimen and two other Styrofoam[®] cup segments were placed close to the light source.

Figure 2-23: Auto-montage lighting



Light from surrounding sources was minimized by pulling the blinds and turning off the fluorescent lights in the room. With the fluorescent lights on the images were tinted in an unnatural blue and possessed unwanted glare. Slide-mounts were placed on two petri-dishes, raising the slide above the microscope stage. Light produced from the microscope base and light from the fiber-optic cables was reduced to a very low level (setting one or two). Styrofoam© cups were not used to diffuse the light; however, it was essential that the room was otherwise dark.

Auto-montage is the process of taking multiple photographs each at a different focal length, removing all of the elements of each photograph that are not in focus, and putting the parts that are in focus into a single image. Syncroscopy© Auto-montage software performs this process automatically. Once the specimen was positioned in the camera's view, a number of choices were made which affected the final product. The camera shutter speed was set at two to four seconds. A slow shutter speed correlates with an increase in the exposure time for each photograph, resulting in an increased amount of light allowed into the camera. A slow shutter speed allows for the use of less light thus reducing glare. One disadvantage of using a slow shutter speed is it decreases the image contrast, or the tonal difference between the strongest

black in the photograph and the strongest white, and it is important to maintain strong contrast when emphasizing cuticle sculpture.

A second variable was the number of photographs taken in the auto-montage process. As a general rule, twenty-five photographs were used for the images within the key. Variation did occur when photographing relatively flat objects, or when large objects were photographed. Each photograph takes time, so reducing the amount of photographs was important in increasing efficiency. For flat objects, the minimal number of images was taken. Label information was photographed with one or two photos and slide mounted specimens with five photographs. In contrast, large specimens often required thirty or more images to produce a well montaged final image.

Editing Photographs in Adobe Photoshop©

Using a neutral grey background, diffuse lighting, and slow shutter speeds produce adequate photographs for the majority of the specimens and characters. However, the images tend to be high in detail but low in contrast with distracting, jumbled backgrounds. The most difficult specimens to photograph are small, black, and shiny, or yellow with a transparent exocuticle. In both of these special cases the post production process is important as the specimen image itself may be jumbled because the auto-montage software has difficulty correctly identifying what part of the specimen is in focus. All of these imaging problems: low contrast, distracting background, and jumbled pixels were corrected in Adobe Photoshop© prior to incorporating the images into the interactive key. Examples of images before and after editing show the improvements (figure 2-24, 2-25). Again this process was standardized during the creation of the Hymenoptera Family Key to reduce the amount of time allotted to each successful image.

Figure 2-24A: Images before and after post production techniques



Figure 2-24B: Images before and after post production techniques



Initially the background was selected using the magic wand tool at a tolerance set at 10, anti-aliased and contiguous. A higher tolerance tends to select into the specimen image, and a tolerance at less than 10 selects only a few pixels at a time. Once the majority of the background is selected corrections were done using the lasso tool. The image edge was observed at 200%, looking for areas where the magic wand did not select close enough to the specimen or where it

selected part of the specimen instead of the background. Wings are often critical areas for close examination of the magic wand selection.

Once the entire background was selected the contrast is removed from the background, which is then darkened slightly. From the Adobe Photoshop[©] menu

<u>Image>Adjustments>Brightness/Contrast</u>, option was chosen and a sliding control bar appeared. The Contrast sliding bar was moved to the -100 position. The brightness bar was moved from a central 0 to between -10 and -2. After the background was removed the Gaussian blur filter was applied to the background from the menu <u>Filter>Blur>Gaussian Blur</u>. The blur radius was set at 3.1, which was generally sufficient. Hairy specimens, such as many Apoidea and Vespoidea, required a lower radius setting of 2.0.

The specimen image was then selected from the menu 'Select>Inverse' and its contrast increased from the menu <u>Image>Adjustments>Auto Contrast</u>. The entire image was then deselected from the menu <u>Select>Deselect</u>. At this point specific corrections were made on the specimen image using the Clone Stamp Tool. This tool allows an area of pixels to be sampled and then replace pixels in another area. It was useful in covering areas of the specimen image that were distorted during the process of auto-montage or removing dust particulates (figure 2-26). The brush size was most effective when it was set at a small size of around 4 pixels.



Figure 2-25: Clone stamp tool

A number of the Symphyta, Proctotrupoidea and Apoidea were too large in size to photograph with a single photo. These specimens were spliced together manually after

photographing all of the different areas of the specimen (figure 2-27). After the specimen is reconnected in Photoshop[©] the same editing protocol was used as for the smaller specimens.



Figure 2-26: Process of reconnecting a large specimen

For wing photographs color was removed and the contrast was increased to emphasize the wing venation. Color was removed by desaturating the image, not changing the mode from RGB to Greyscale, because a Greyscale image becomes incompatible with a RGB or color image document. To desaturate an image from the menu choose <u>Image>Adjustments>Desaturate</u>. Contrast is increased after desaturation, and the background of wing images is often removed to a very light grey in order to emphasize the wing shape.

Images used to illustrate characters and taxa were set at approximately 760 pixels X 527-560 pixels; the maximum size image that could fit in its entirety on a mid-size monitor. Character state images were placed in a single Photoshop[©] document of that size and arranged on the page so they were easily viewed and the overlays were easily placed on top of the images. During this process the images were often reduced in size. The original corrected image was saved as a Photoshop[©], '.psd),' document in a separate folder to archive the original pixel information. The colors used for the arrows, background and text were standardized in the Hymenoptera Family Key using the ANAP color system for illustrations and graphics. The

background color for character images was ANPA 737-6 AdPro, text/arrows were ANPA 755-O AdPro or true black. This color system was chosen for its availability in Adobe Photoshop[©] and its intended use for images on computer screens. The background blue/green color was chosen to increase the keys visual appeal (Hall & Hanna, 2004).

In conclusion, the primary purpose for creating images for INTKEY is to illustrate taxa and characters. Although the photographic guidelines described here were maintained to increase efficiency in the photographic process, they were manipulated on an individual photograph basis to increase image quality and effectiveness in the Hymenoptera Interactive Key. Each image must illustrate the character in the described state in order to be informative. Ideally, images illustrating character states should illustrate not only the most obvious examples of that state, but also the intermediate conditions. Sometimes photographs, although generally the preferred illustrative method for the Hymenoptera Key to Families Project were used. This occurred when a character is difficult to photograph, or an appropriate specimen is not available. In these circumstances drawings are used from the Henri Goulet and John Huber text, *Hymenoptera of the World: An Identification Guide to Families*.

Chapter III: Results

Interactive Key Example

The project resulted in a series of Hymenoptera Interactive Keys using the INTKEY program written by (Dallwitz et al., 1993). The keys contain 145 taxonomic divisions, 286 characters, 600 photographs, and 40 line drawings. The line drawings, primarily drawn by S. Rigby, come from *Hymenoptera of the World: an Identification Guide to Families*. Scanning electron microscope images are from Matt Buffington and Fredrik Ronquist. The keys are an expandable and easily updatable foundation for more detailed keys to the order; thus, the majority of the information found within the keys is attached in matrix form at the end of the document.

An INTKEY example of the superfamily Proctotrupoidea is attached as an 'Intkey.ink' file and as a series of screen shots with descriptions of the keys function. The key is intended to illustrate the program not as a final product. In order to view the key a small program must be installed on a computer running Windows 95 thru Windows XP. The program may be obtained here. Save the '.exe file' to your computer's desktop, double click on the 'intkey.exe' file and follow the installation instructions. It may be necessary to restart the computer before INTKEY can run properly. Once the INTKEY program is installed on the computer any INTKEY interactive keys may run from that machine. The example INTKEY to the Proctotrupoidea is found here. The key will open automatically in the Web browser.

The Interactive Key to the Proctotrupoidea begins with a splash page that introduces the key.



Figure 3-1: Hymenoptera Interactive Keys; Proctotrupoidea splash page

Figure 3-2: Hymenoptera Interactive Keys; Proctotrupoidea opening page



After the initial splash page, a typical INTKEY window opens, with a list of characters on the top left column and a list of taxa on the top right column (figure 3-2). This window opens with the characters listed in the Best Order. This ranks the characters based on their effectiveness in keying taxa. The two large buttons at the top left are linking buttons for the Contents files and the Hymenoptera Glossary. The Contents text file lists the introduction, acknowledgements, and references for the particular interactive key. The Hymenopteran Glossary button is a web link to the Hymenopteran Glossary. These buttons are modified in the Hymenoptera Interactive Keys using the 'toolbar. Imp' directive file in DELTA. The Hymenopteran Glossary is derived from the glossary contained within the text *Hymenoptera of the World: an Identification Guide to Families*, edited by Michael J. Sharkey and John Huber. The reproduced drawings of key terms are also taken from this text.



Figure 3-3: Hymenoptera Interactive Keys; Proctotrupoidea INTKEY integer character

When the character "How many flagellomeres does the antenna have?" is double clicked, the associated image file is retrieved from the image folder on the web (figure 3-3). Overlays associated with the character are a text box for entering the number of flagellomeres, a character description and explanatory text boxes describing what is required of the user.

The number of flagellomeres of the user's specimen is entered in the cell and the **OK** button is pressed, eliminating all of the taxa outside of that range.

Figure 3-4: Hymenoptera Interactive Keys; Proctotrupoidea INTKEY after the selection of

character "How many flagellomeres does the antenna have?"



The **Used Characters** are listed on the bottom left of the screen and the **Eliminated Taxa** are listed on the bottom right column of the screen (figure 3-4). The user may change their mind about character choice at any time during the keying process by double clicking a used character and modifying the character state choice.

Figure 3-5: Hymenoptera Interactive Keys; Proctotrupoidea INTKEY comparison of taxa by double clicking the taxon name



Taxon images are double clicked to show associated images. At any time during the keying process the user may compare habitus images of taxa (figure 3-5). Multiple images may be entered in the habitus window for to facilitate the comparison of taxa.

Figure 3-6: Hymenoptera Interactive Keys; Proctotrupoidea INTKEY unordered multistate character



Any character may be clicked in the list of characters, allowing the user to choose characters specifically for the specimen they intend to identify. For example, an unusual morphological character such as a strong antennal shelf quickly keys a specimen to Diapriidae (figure 3-6) immediately. This is an example of an unordered multistate character, which gives the user discrete choices. The specimen may have an antennal shelf or not. When the character state "yes" is chosen, the yes tab becomes black, indicating to the user which choice he/she has selected.



Figure 3-7: Hymenoptera Interactive Keys; Proctotrupoidea INTKEY complete identification

When identification is reached (figure 3-7) only one taxon will remain in the **Remaining Taxa** field and a help button appears in the **Best Characters** field. The numbers beside the eliminated taxa represent the number of times the user chose a character that eliminates that taxon. The higher the number, the more confidence the user may have of a correct identification of the specimen. Again, the identified taxon habitus images may then be viewed by double clicking the taxon name, and these images compared to the specimen the user wishes to identify.

Difficulties and Key Challenges

The Hymenoptera Family Keys are at varying levels of quality because of differences in collaborator involvement, specimen availability, inherent difficulty of the superfamily, and time constraints. The keys with strong collaborator involvement tend to be better. These include the family level keys: Proctotrupoidea, Chrysidoidea, and Symphyta. Keys without strong collaborator involvement, such as the superfamily key, are less efficient because the matrix is not

as complete, and the user will usually be required to go through a greater number of characters to reach identification than would be the case if the matrix were complete. Coding characters for the entire family was difficult because of the great variation among many of the families; thus, characters were not coded for families if there was low confidence in character state choice for that family. Some taxa and characters are not illustrated due to unavailability of specimens.

Keys have been modified from their original dichotomous keys to varying degrees. The Superfamily Key, Platygastroidea, and Ichneumonoidea remain closest to the original dichotomous key source material, as most of the characters, character states and taxonomic divisions remain unchanged. The keys have varying levels of probable success for reaching a correct identification for a specimen. The Chrysidoidea, Symphyta, Proctotrupoidea, Ceraphronoidea, Evanioidea and Platygastroidea keys are successful under preliminary trials with unknown specimens; however, they have not been extensively tested The Apoidea key is the most inefficient of the keys due to the uncertain phylogeny of the superfamily and a high degree of convergence in various families, for example, many of the external keying characters for the families Megachilidae and Apidae are lost in the cleptoparasitic family members (Michener, 2000), resulting in a great number of exceptions to each family. These exceptions are separated in the key primarily using mouthpart morphology.

Chapter IV: Future Prospects and Considerations

Summary of Future Prospects

The Interactive Key to the Hymenopteran Families is intended as an initial effort to construct an interactive key to the families of the order based on the current phylogenetic hypotheses. The keys will be integrated into the Hymenoptera Assembling the Tree of Life Project (HymAToL), supported by the National Science Foundation. The HymAToL project intends to continue updating and improving the keys based on explorations into the phylogeny of the order, and will ultimately include an interactive key to the families of the Chalcidoidea. The level of detail within the keys will be improved by including more images illustrating the taxonomic diversity, using specific names for taxon images, adding detailed notes and character images associated with problematic character states, and images illustrating exceptional taxa. As part of ongoing collaboration among Hymenopterists, the keys will link at the family level to existing keys to genera, web sites of researchers actively investigating a taxon, and species pages. The image files associated with the key will be uploaded to an online image database where they will be available to the public. Morphbank (<u>http://www.morphbank.org</u>) is a primary consideration due to its use in the HymAToL project. The public availability of the keys will be announced at professional meetings and through list-servs such as ENTO-L and TAXICOM.

Presently the interactive keys require the installation of the program INTKEY on the user's computer in order to view the keys, potentially limiting public access. Commonwealth Scientific and Industrial Research Organization (CSIRO) plans to release a web-native version of INTKEY (Steve Shattuck, personal communication) and the prospect of transferring the interactive keys will be evaluated at that time. Eventually the use of online image databases, such as Morphbank, may replace much of our need for printed material and will connect files directly from these databases to interactive keys for use by researchers, educators and entomological enthusiasts.

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Chapter V: DELTA Matrices

The matrices are in Excel[©] format. The taxa are linked to habitus illustrations found within the key. If multiple habitus images exist for a taxon, the link corresponds to the first image on the list. Characters are listed in the matrices by consecutive numbers: 1, 2, 3. etc. These numbers are linked to the character description and character states as generated by the DELTA chars file. Character state images and taxon images, if present, open in a new window and can be closed by closing the browser window.

The matrices are:

Apoidea
Ceraphronoidea
Chrysidoidea
Cynipoidea
Evanioidea
Ichneumonoidea
Platygastroidea
Proctotrupoidea
Superfamily
Symphyta
Vespoidea

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Membership

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