

The diversity and identification of eulophid parasitic wasps (Hymenoptera: Chalcidoidea: Eulophidae) on *Phyllocnistis labyrinthella* (Lepidoptera: Gracillariidae) from Västerbotten, Sweden

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Caterpillars of the mining micro-moth *Phyllocnistis labyrinthella* feed on leaves of aspen (*Populus tremula*) and are often parasitized by eulophid wasps. The parasitoids are a potential important cause of death for the miners. During 2009-2011 we collected mined leaves from an experimental stand of aspen trees in Västerbotten. Adults emerged from 17-35 percent of the mines and of these every second to third specimen appeared as wasps. These wasps represented seven species of eulophid parasitoids of which three were new to Västerbotten: *Chrysocharis nitetis* (Walker), *Cirrospilus diallus* (Walker) and *Cirrospilus pictus* (Nees). We include an identification key that may be used to identify these eulophid species.

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The mining moth *Phyllocnistis labyrinthella* is present in large parts of Sweden where it attacks leaves of aspen (*Populus tremula*) (Fig. 1) (Albrechtsen et al. 2010). In a similar North American system Wagner et al. (2008) demonstrated how colonisation by *Phyllocnistis populiella* negatively affects growth, changes physiology, and shortens leaf longevity of its host, American aspen (*Populus tremuloides*). Parasitoids naturally attack the miners and potentially regulate their numbers, as demonstrated for other insect species (Quicke 1997, Grissell & Schauff 1997). This ability has been used commercially - mainly in closed systems like greenhouses - to

control insect pests in a biological way (Smith 1996, Pilkington et al. 2010). In open natural systems the role of parasitoids is more complex. Parasitoid composition and abundance vary in both space and time (Sundby 1957, Askew and Coshan 1973), and recent reports have evidenced that plants may indirectly defend themselves by the presence of extra floral nectaries (Young et al. 2010) or by emitting volatile compounds (Danner et al. 2011) which may both attract body guards in the form of parasitoids or predators.

As part of our project about the role of plant direct and indirect defences in Swedish Aspen we became interested in the diversity and abundance



Figure 1. An aspen leaf mined by the micro-lepidopteran *Phyllocnistis labyrinthella*. Mined leaves were collected from a common garden with 450 young trees in Sävar through 2009-2011 to study the load of parasitoids that emerged from the pupae. Every second to third insect that emerged from the pupae were parasitoids belonging to one of seven eulophid species.

Ett asplblad med en bladmina av aspsaftmal (*Phyllocnistis labyrinthella*). Minerade blad samlades in från ett bestånd med 450 unga träd i Sävar under 2009 till 2011 för att studera i vilken omfattning parasitoider kläcktes från puporna. Var andra till tredje vuxen insekt var en parasitoid tillhörande en av sju eulophidarter.

of parasitoids. Parasitic wasps form a morphologically very diverse group of the order Hymenoptera with a way of life in common. These wasps develop as larvae on other arthropods, usually other insects, their so called hosts. The hosts are with few exceptions eventually killed by their parasitoid(s). Traditionally parasitic wasps are classified as an infra-order (Parasitica, Table 1) under the sub-order Apocrita (wasps with a waist), and are represented by eight superfamilies in Sweden. The adult females of these wasps oviposit inside, on, or next to their hosts and the emerging parasitoid larva will feed on the host throughout its development. More or less every insect species is known to be parasitized by a parasitic wasp during some part of its life cycle, and parasitoids therefore regulate populations of other insect species (Quicke 1997, Grissell & Schauff 1997). Some species are generalists and parasitize several different hosts. Others are more specialized with a narrow host range. In both cases the parasitoids are primary consumers that develop on the host, so called primary parasitoids. Some parasitoids develop on other parasitoids that in their turn develop on the host, and these parasitoids are called secondary parasitoids or hyperparasitoids. The many niches that parasitoids successfully occupy are

Table 1. Parasitica (infraorder: parasitic wasps) and their representation in Sweden, by number of families and species. Number in parenthesis refers to the number of levels in the subordinate category Superfamilies under infraorder and families under Superfamilies. TaxonID refers to the code in Dyntaxa, the Swedish taxonomic database : <http://www.artdata.slu.se/dyntaxa/>.

Parasitsteklar (infraorder: Parasitica) ordnade efter överfamilj, antal familjer och arter. Siffror inom parentes refererar till antal grupper i den underordnade kategorin: överfamiljer under infraordning, och familjer under överfamilj. TaxonID refererar till respektive kod i Dyntaxa, den svenska taxonomiska databasen : <http://www.artdata.slu.se/dyntaxa/>.

TaxonID	InfraOrder	TaxonID	SuperFamily	#Species	TaxonID	Family, Swedish Name	#Species
		2002987	Ceraphronoidea	(2) 76	2001153	Aphelinidae Växtlussteklar	38
2001133	Parasitica (8)	2002985	Chalcidoidea	(14) 1822	2001146	Chalcididae Bredlärssteklar	18
		2002984	Cynipoidea	(3) 261	2001152	Encyrtidae Sköldlussteklar	192
		2002983	Evanoidea	(3) 12	2001158	Eulophidae Finglanssteklar	565
		2002988	Ichneumonoidea	(2) 3953	2001151	Eupelmidae Hoppglanssteklar	16
		2003743	Mymarommatoida	(1) 1	2001147	Eurytomidae Kragglanssteklar	97
		2003744	Platygastridae	(1) 257	2001145	Mymaridae Dvärgsteklar	38
		2002986	Proctotrupoidea	(4) 382	2001149	Ormyridae Kägglanssteklar	6
					2001150	Perilampidae Gropglanssteklar	15
					2001157	Pteromalidae Puppglanssteklar	691
					2001154	Signiphoridae Långklubbsteklar	2
					2001155	Tetracampidae Raggsteklar	9
					2001148	Torymidae Gallglanssteklar	117
					2001156	Trichogrammatidae Hårstrimsteklar	18

one potential reason why parasitic wasps have become evolutionary successful and form a very species-rich group. The Swedish Taxonomy Database, Dyntaxa, lists 6764 species of parasitic wasps in Sweden, including 1822 species of the superfamily Chalcidoidea (Table 1). This group is divided into 14 families present in Sweden of which the Eulophidae comprises mainly small wasps that develop for example in leaf mining insects, gall makers, and on caterpillars (Sundby 1957, Hansson 1987), and with 537 species recorded from Sweden (Dyntaxa, March 2011, Table 1).

The most comprehensive list of Swedish species of the Chalcidoidea is probably the catalogue by Karl-Johan Hedqvist (2003) that documents names and findings in the Swedish provinces from a 50 year collection of parasitic wasps, based on 50.000 specimens. Worldwide, Chalcidoidea approaches 19.000 described species, a likely underestimate of a group which is suggested to contain 3 to 6 times more undescribed species (Grissell & Schauff 1997).

Swedish aspen (*Populus tremula*) is associated with a species rich community of arthropods, mainly characterised by specialist herbivores (Albrechtsen 2005, Robinson et al 2012). *Phyllocnistis* leaf mining moths are highly abundant in mainly mid and northern Sweden, whereas it is quite scarce in the south (Albrechtsen et al. 2010). The present study is based on emerging insects from *Phyllocnistis*-mined leaves collected in our common garden in Sävar, Västerbotten during 2009 to 2011. Here we report the relationship between moth damage levels and parasitoid success and diversity. We also present an identification key which is focused on the parasitoids relevant for this system.

Material and Methods

Study System

Phyllocnistis labyrinthella (Lepidoptera, Gracilariidae) is a common leaf miner in Sweden with spatial large scale variation in abundances (Albrechtsen et al. 2010). Another species, *Phyllocnistis unipunctella*, usually co-occurs with *P. labyrinthella* but is only responsible for a fraction of the mines when evaluated through studies of emergence (Sundby 1957), or on the basis of mine morphology identification (Albrechtsen

Handling of Eulophids

Direct mounting

Freezer -20°C (10 min)

Storage

80% ethanol sealed with lock, keep dark and cold (mount as soon as possible because the animals will degenerate in alcohol)

Dehydration

- a. Critical Point Drier
or
- b. 96% ethanol (2x15min)
100% ethanol (15min)
X% acetone (5-10min)
Dry on tissue (5-10min)

Mount work

Pin size 4

Glue or transparent nail polish

Paper label potentially pointed (max 8x12 mm)

Attach wasp's right side just above the middle right leg (mesothorax), spread wings and legs to display details. Paper labels (8x12



Mounted insect

- a. Collection details: place, date, collector
- b. Identification details: species, family
- c. Ecological details: plant genotype and host insect

Box 1. To obtain the best specimens to identify parasitoids that emerge, the material should be processed with care and quickly. If mounted while the insect is still soft it may be arranged to display important characters for identification. For the sampling in 2010 we used the direct mounting method. Insects in alcohol shall be kept dark and cold, but storage for a longer period of time should be avoided due to the deterioration of the specimens.

Parasitsteklar bör hanteras så snabbt och försiktigt som möjligt för att få bra exemplar för artbestämning. Om man monterar insekten när den fortfarande är mjuk kan kroppen arrangeras så att viktiga karaktärer syns bra. Under 2010 och 2011 använde vi oss av direkt montering. Djur i sprit skall förvaras mörkt och kallt, men förvaring i sprit under en längre tid bör undvikas eftersom djuren då förändras negativt.

et al. 2010). The variation in parasitoid abundance and diversity is a potential key to understand the temporal and spatial variation in the population patterns of *Phyllocnistis* species on aspen (Sundby 1957). As described in Albrecht-

sen et al. (2010) *Phyllocnistis* species appear to be more abundant in mid Sweden, where aspen trees may appear silvery from their high loads of the labyrinth-shaped mines on the leaves (Fig. 1). In northern Sweden the densities are comparatively lower but mines occur frequently in the aspen stands. In the south of Sweden the abundance and density of these miners have been suggested to be comparatively lower (Albrechtsen et al. 2010).

Phyllocnistis moths are mainly parasitized by eulophid wasps (Sundby 1957), and different genera of these parasitoids have different biologies. Species belonging to the Entedoninae

(*Chrysocharis* belongs here) are usually koinobiont endoparasitoids. Koinobionts allow their host to continue to live and feed after the initial parasitisation, as opposed to idiobionts that paralyzes their hosts permanently. Koinobionts may initially delay their own development so that the host builds up enough biomass for the parasitoid to go through with its development successfully, and they may even speed up the development of their host (Dziurzynski, 1961). Species of the Eulophinae are ectoparasitoids and usually idiobionts, that prevent further development of their host after the initial parasitization. Eulophinae includes 26 genera in Sweden (Dyntaxa, June 2011) of which three have relevance for this study: *Cirrospilus*, *Pnigalio* and *Sympiesis*.

Table 2. Lifestable showing emergence success of the moth *P. labyrinthella* and its parasitoid wasps. Data were obtained through studies of mined aspen leaves from the SwAsp Sävar common garden (2009-2011). "N" = number of individuals in a developmental stage, "Dead" = individuals that did not advance to the following stage, "Mortality" = the ratio Dead/N, "Global success" = the ratio of survivors in a stage (N_x) relative to the original number of collected mines N_{mines} , using the following formula: N_x/N_{mines} .

Tabell 2. Demografisk tabell med minor av aspssåftmal och deras kläckningsresultat, insamlade 2009-2011 i Sävar, på aspar från SwAspbeståndet. N = antal individer i respektive stadium, "Dead" = individer som inte fortsatte till nästa utvecklingsstadium, "Mortality" = kvoten Dead/N, "Global success" = sannolikheten att utvecklas till ett stadium N_x i relation till ursprungliga antal minor N_{mines} , beräknad som N_x/N_{mines} .

Year/Stadium	N	Dead	Mortality	Global success
Year: 2009				
Mines	2457	1006	0.41	1.00
w pupal chamber	1451	1040	0.72	0.59
Emerged insects	411	197	0.48	0.17
			(parasitoids: 0.08)	
Moths	214			0.10
Year: 2010				
Mines	1386	507	0.37	1.00
w pupal chamber	879	530	0.60	0.63
Emerged insects	349	105	0.30	0.25
			(parasitoids: 0.08)	
Moths	244			0.18
Year: 2011				
Mines	1591	430	0.27	1.00
w pupal chamber	1161	612	0.53	0.73
Emerged insects	549	165	0.30	0.35
			(parasitoids: 0.10)	
Moths	384			0.24

Collection

The peak occurrence for collection of mined leaves varies within a year and from year to year (Albrechtsen et al. 2010). In mid July (22-24 July 2009, 19 July 2010, and 12-13 July 2011), after pupal chambers had developed, the canopies of the SwAsp common garden in Sävar (Luquez et al. 2008, Albrechtsen et al. 2010) were carefully inspected and every detected mined leaf was collected. In 2009 leaves were collected from all trees - ca 450 individual trees in total. In 2010 only a subset of the trees were collected - a total of ca 150, and the same trees were re-sampled in 2011. To let the insects emerge, leaves with *Phyllocnistis* pupal chambers were kept at room temperature. The first season the leaves were gathered into large emergence containers and the insects were sorted out from the leaf material after they had emerged. In 2010 and 2011 individual leaves were kept separately in 2 ml sealed glass tubes, in which emergence of animals was recorded on a daily basis. When an adult appeared in a tube, the tube (including its content) was taken to the freezer (-20°C) for 10 minutes and then kept at room temperature for further processing (Box 1).

Results

Morphological inspections of the mines and subsequent identification of the emerged moths confirmed previous observations from the SwAsp field based on mine morphology, that ca 99 % of the moths were *Phyllocnistis labyrinthella*

and the rest were *P. unipunctella* (Albrechtsen et al. 2010). Seventeen, 25 to 35 % of the mines respectively, resulted in emerging insects, of which 30-48 percent were parasitoids. Thus, when calculated on the basis of the total number of collected mines only 10-24 % emerged successfully as moths (Table 2). Out of 197 (2009), 105 (2010), and 165 (2011) emerged parasitoids, the specimens represented seven, four, and six species, respectively. All parasitoids belonged to the Eulophidae and included both endoparasitic species within *Chrysocharis*, and ectoparasitic species belonging to *Cirrospilus*, *Pnigalio* and *Sympiesis*. To facilitate the identification of these species a key was made (Box 2). Four of the parasitoid species agreed with findings by Sundby (1957) and Hedqvist (2003) (Table 3) and three were new to Västerbotten. The sex-ratio was also skewed in favour of females with 60 to 100 percent females, depending on species and year. A similar female bias was reported by Sundby (1957).

Discussion

In an effort to understand the relationship between moth damage levels and parasitoid abundance and diversity we studied parasitic wasps on *P. labyrinthella* in a common garden of aspen in Sävar, Västerbotten, during three years. Of

the total number of mined leaves between eight and ten percent emerged as parasitoids, and the moth success varied between ten and 24 percent depending on year. A three year period is too short a time period to characterise the dynamics in details, however we may conclude that between half (2009) and a third (2010 and 2011) of the insects that emerged were parasitoids, and that the parasitoids thus were a considerable mortality factor at the pupal stage. The moths were parasitized by eulophids only, which agrees with previous information (Sundby 1957, Noyes 1982). A major part (65-83 percent) of the mortality of the moths thus occurred during immature stages. We only included mines with pupal chambers in our emergence studies, and parasitoids that arrest juvenile host stages were thus not part of our material. This is a major difficulty when studying these relationships. If mines are harvested early to study parasitoids in the larval stages, developing larvae may be cut off from their feeding supply, whereas when harvested late only parasitoids that emerge in synchrony with the moths will be included.

We identified seven parasitoid species in our material of which three species were new for Västerbotten. Our second most common species, *Cirrospilus diallus*, was not reported by Sundby (1957) who identified 16 species from sev-

Table 3. **Number of specimens** and (*percentage females*) of identified eulophid parasitoids in SwAsp, Sävar, 2009-2011. Previous reports from Västerbotten (Vb) by Hedqvist (2003), and from Norway with names used by Sundby (1957). Synonym names are verified with the help of the "Universal Chalcidoidea Database" <http://www.nhm.ac.uk/jdsml/research-curation/research/projects/chalcidooids/>.

Antal individer (i fet stil) och *andel honor i %* (kursiv inom parentes) av finglanssteklelarter som parasiterar på aspssaftmal, insamlade från SwAsp, Sävar, (2009-2011). Fynden jämförs med tidigare uppgifter från Västerbotten av Hedqvist (2003), och från Norge med de namn som användes av Sundby (1957). Artsynonymer är kontrollerade med hjälp av "Universal Chalcidoidea Database" <http://www.nhm.ac.uk/jdsml/research-curation/research/projects/chalcidooids/>.

Species	Sävar			Previously reported from	
	2009	2010	2011	Västerbotten	Norway
<i>Chrysocharis nephereus</i> (Walker)	137 (77)	47 (83)	51 (53)	Yes	<i>Epilampsis gunholdi</i> (Del), <i>Epilampsis tadici</i> (Del)
<i>Chrysocharis nitetis</i> (Walker)	2 (100)	0	0	Not reported	<i>Epilampsis boops</i> (Thoms)
<i>Cirrospilus diallus</i> (Walker)	26 (96)	26 (65)	55 (95)	Not reported	<i>Not reported</i>
<i>Cirrospilus pictus</i> (Nees)	5 (80)	0	0	Not reported	<i>Cirrospilus pictus</i> (Nees)
<i>Cirrospilus vittatus</i> (Walker)	4 (100)	4 (100)	1 (100)	Yes	<i>Cirrospilus vittatus</i> (Walker)
<i>Pnigalio pectinicornis</i> (L.)	2 (100)	0	1 (100)	Yes	<i>Eulophus pectinicornis</i> (L)
<i>Sympiesis sericeicornis</i> (Nees)	9 (88)	16 (88)	52 (54)	Yes	<i>Sympiesis sericeicornis</i> (Nees)
<i>Tetrastichinae</i> indet.	2	0	1	-	-
Unidentified	10	12	4	-	-
Sum	197	105	165		

Box 2. Identification key to the euploids encountered with *Phyllocnistis labyrinthella* in Västerbotten. For explanation of morphological terms see Fig. 2.

Bestämningstabell till de finglanssteklar som påträffats på aspsaftmal i Västerbotten. Namnen på vissa kroppsdelar förklaras i Fig. 2.

1. Head, mesosoma and/or gaster (i.e. at least one of these body parts) bi-coloured, partly metallic, partly yellow to brown; scutellum with two fine lateral grooves2

– Head, mesosoma and gaster completely metallic; scutellum without lateral grooves4

2. Scutellum bicoloured with longitudinal stripes in metallic and yellow*Cirrospilus vittatus*

– Scutellum completely dark3

3. Propodeum with complete or incomplete lateral carinae*Cirrospilus diallus*

– Propodeum without lateral carinae*Cirrospilus pictus*

4. Forewing submarginal vein dorsally with more than two setae; antennal flagellum with six segments5

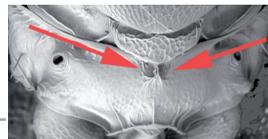
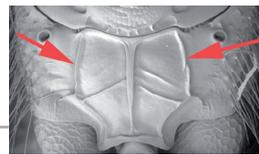
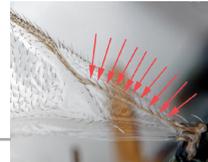
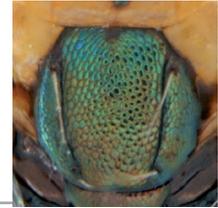
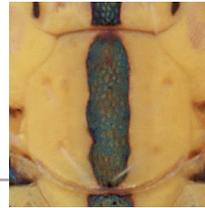
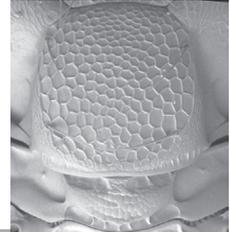
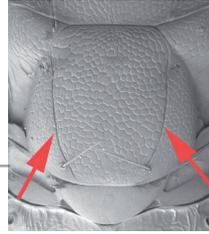
– Forewing submarginal vein dorsally with two setae; antennal flagellum with five segments6

5. Median propodeum with lateral longitudinal carinae*Pnigalio pectinicornis*

– Median propodeum without lateral carinae*Sympiesis sericeicornis*

6. Anteromedian propodeum with two small foveae*Chrysocharis nephereus*

– Anteromedian propodeum with strong carinae*Chrysocharis nitetis*



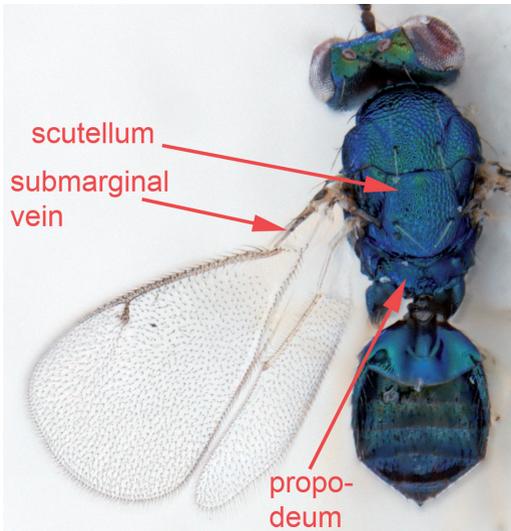


Figure 2. Some morphological parts of eulophids useful for identification (see Box 2).

Några kroppsdelar på finglanssteklar som är användbara för artbestämning (see Box 2).

eral thousand specimens, many of which have also been reported from Västerbotten, Sweden (Hedqvist 2003). In our study we further found that the number of species was positively related to the number of collected specimens of parasitoids whereas the rate of parasitism was close to constant. *Chrysocharis nephereus* (Walker) was always abundant in Sävar. This species was also present in Sundby's comprehensive surveys in Norway (1957) under two names: *Epilampsis gunholdi* (Delucchi) and *E. tadici* (Delucchi), but not as a dominant species. *C. nephereus* is a generalist that may attack miners on several tree species, for example birch, rowan and willows (Askew and Coshan 1973), all of which are trees that occur naturally in Västerbotten. Generalist parasitoids are not closely coupled with a single host, but through apparent competition the more abundant host may be preferred and we would thus expect the relationship between the generalists and their hosts to fluctuate more than that of specialists. During the three study years, *C. nephereus* showed higher abundances and more

relative fluctuation than any of the other parasitoids, although *C. diallus* and *S. sericeicornis* also expressed significant dynamics.

Plants defend themselves in several ways, and through indirect defences they may attract "body guards" in the form of parasitoids and predators, that reduce the number of damaging herbivores. Volatile emissions thus function as wounding signals by which plants make parasitoids aware of herbivore hosts and extrafloral nectaries allure ants to patrol stems and leaves and keep them free of many phytophagous arthropods. Aspen employ both of these indirect defence mechanisms: they emit particular volatiles in response to chewing caterpillars (Danner et al. 2011) and their leaves have extra floral nectaries that may attract natural enemies (Young et al. 2010); but it still remains to be demonstrated that this has any impact on the dynamics of herbivores on *P. tremula* in general and in particular if it reduces damage by *P. labyrinthella* moths. Closer studies of the phenology of the parasitoids in the field with particular interest in moth-mortality during the juvenile stages would be valuable future supplements to further understand the regulatory potential of moths in this system.

As a first step to study the effect of parasitoid wasps on *P. labyrinthella* in aspen stands we provide a key for their identification. For future identifications this key will be used and unidentified specimens send to expert determination.

Tack

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Sammanfattning

Aspsaftmal (*Phyllocnistis labyrinthella*) som minerar i blad på asp (*Populus tremula*) (Fig. 1) parasiteras av finglanssteklar (Chalcidoidea: Eulophidae). Dessa parasitsteklar kan potentiellt reglera storleken på malarnas populationer. För att undersöka parasitstekelfaunans sammansättning och deras påverkan på aspsaftmalen kläcktes fjärilar och parasitsteklar fram ur bladminor. Minorna insamlades 2009-2011 i ett experimentellt bestånd av aspar i Sävar i Västerbotten. Ur 17-35% av minorna utvecklades vuxna insekter och av dessa var mellan 30-48 % parasitoider, samtliga finglanssteklar. Steklarna identifierades och artsammansättningen sammanföll i viss mån med tidigare uppgifter från Norge och Norra Sverige, men tre av arterna var nya för Västerbotten: *Chrysocharis nitetis* (Walker), *Cirrospilus diallus* (Walker) och *Cirrospilus pictus* (Nees). Vi inkluderar en bestämningsnyckel (Box 2) med vilken arterna vi funnit på aspsaftmal kan identifieras.