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Determinants of parasitoid communities of willow-galling sawflies: habitat overrides physiology, host plant and space

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Abstract

Studies on the determinants of plant–herbivore and herbivore–parasitoid associations provide important insights into the origin and maintenance of global and local species richness. If parasitoids are specialists on herbivore niches rather than on herbivore taxa, then alternating escape of herbivores into novel niches and delayed resource tracking by parasitoids could fuel diversification at both trophic levels. We used DNA barcoding to identify parasitoids that attack larvae of seven *Pontania* sawfly species that induce leaf galls on eight willow species growing in subarctic and arctic–alpine habitats in three geographic locations in northern Fennoscandia, and then applied distance- and model-based multivariate analyses and phylogenetic regression methods to evaluate the hierarchical importance of location, phylogeny and different galler niche dimensions on parasitoid host use. We found statistically significant variation in parasitoid communities across geographic locations and willow host species, but the differences were mainly quantitative due to extensive sharing of enemies among gallers within habitat types. By contrast, the divide between habitats defined two qualitatively different network compartments, because many common parasitoids exhibited strong habitat preference. Galler and parasitoid phylogenies did not explain associations, because distantly related arctic–alpine gallers were attacked by a species-poor enemy community dominated by two parasitoid species that most likely have independently tracked the gallers' evolutionary shifts into the novel habitat. Our results indicate that barcode- and phylogeny-based analyses of food webs that span forested vs. tundra or grassland environments could improve our understanding of vertical diversification effects in complex plant–herbivore–parasitoid networks.

Keywords: community barcoding, enemy-free space, speciation, tritrophic food webs, vertical diversification effects

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Introduction

Studies on food webs consisting of plants, herbivorous insects and insect parasitoids are central to our understanding of the functioning of terrestrial ecosystems as well as the origin of global and local species diversity (Singer & Stireman 2005; Kitching 2006; Abrahamson & Blair 2008). Plant–herbivore–parasitoid networks are characterized by high species richness and variably specialized trophic interactions (Weiblen *et al.* 2006; Eveleigh *et al.* 2007; Cagnolo *et al.* 2011), and this immense complexity poses formidable challenges for research (Hawkins 1994; Forister & Feldman 2011; Hrcek *et al.*

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2013). A major complication is that both herbivores and parasitoids tend to be small and difficult to identify morphologically (Smith *et al.* 2008; Kaartinen *et al.* 2010; Fernandez-Triana *et al.* 2011). Fortunately, molecular-genetic methods provide increasingly efficient tools for species identification from all insect life stages, and rapid screening of community structure based on DNA barcode sequences allows improved estimation of interaction strengths across trophic levels (Hrcek *et al.* 2011; Smith *et al.* 2011; Wirta *et al.* 2014).

Important insights into the ecology and evolution of plant–herbivore–parasitoid networks can be achieved by studying factors that determine patterns of parasitoid attack across potentially available herbivore species. The parasitoid community attacking a given insect host population is shaped by multiple interacting factors, the most obvious of which are time and space, because plants, herbivores and parasitoids occur as spatially and temporally variable multitrophic metacommunities (Schönrogge *et al.* 1998; van Nouhuys & Hanski 2005; Klapwijk & Lewis 2011; Lohse *et al.* 2012; Stone *et al.* 2012). Species-specific physiological traits and immune responses of herbivores may also limit their suitability for parasitoids, although immune defences are typically not directly observable in natural conditions (Desneux *et al.* 2012; Harvey *et al.* 2012; Condon *et al.* 2014). From an evolutionary viewpoint, the most interesting potential determinant is host ecology, because niche-dependent parasitism could theoretically explain the extreme species diversity of both herbivores and parasitoids: parasitoids that search for victims in specific niches could facilitate shifts to new host plants or resources in herbivores and thereby trigger ecological speciation in the herbivores (i.e. top-down diversification; Gratton & Welter 1999; Singer & Stireman 2005). The resultant increase in ecological and lineage diversity in the herbivores could then open up possibilities for further parasitoid diversification (i.e. bottom-up effects; Stireman *et al.* 2006; Abrahamson & Blair 2008; Forbes *et al.* 2009).

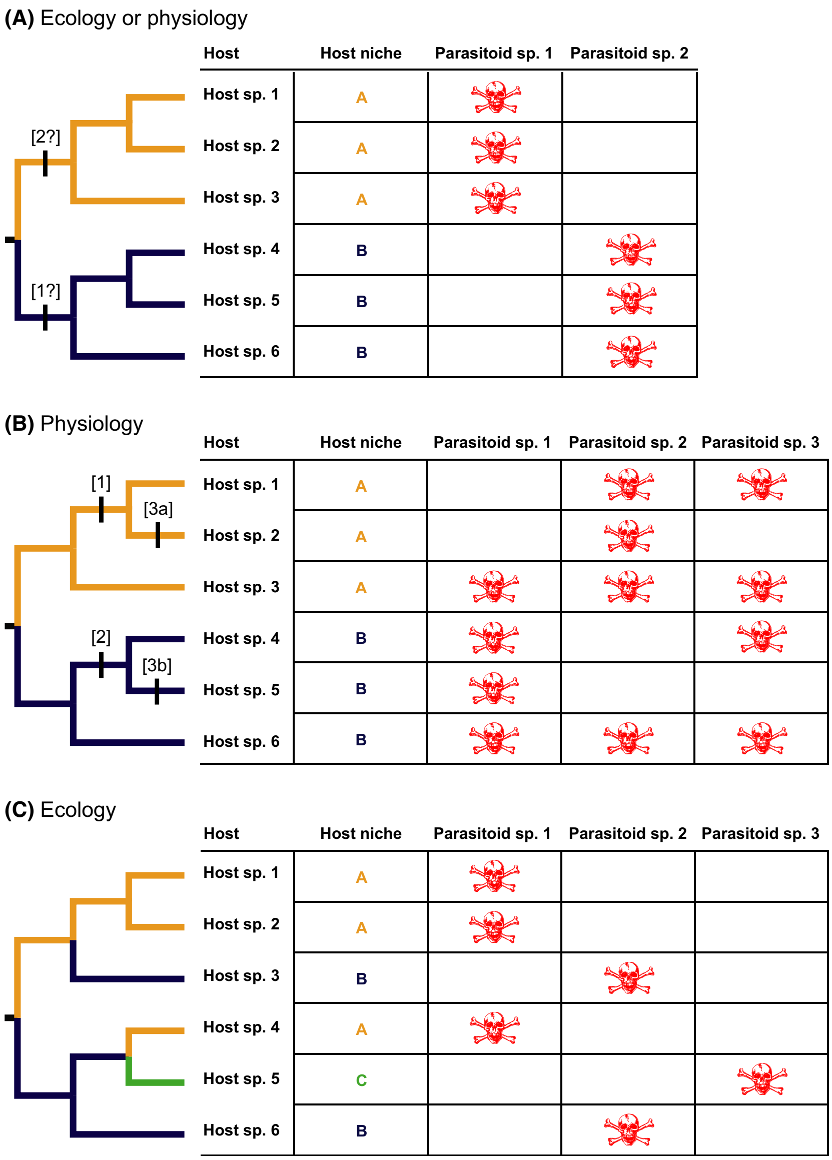
Surveys based on mass rearings of oligo- and polyphagous insects have indeed shown that levels of parasitism can vary across alternative host plants (Price *et al.* 1980; Lill *et al.* 2002; Farkas & Singer 2013). Demonstrating ecologically based parasitism is more complicated in specialists occupying single, distinct niches. In such cases, comparisons across multiple lineages utilizing different resources are needed. However, simply showing that species occupying alternative niches or niche dimensions are attacked by distinct parasitoid communities is not enough to demonstrate that the differences between the groups are *caused* by their ecological features. This is because both physiological defences and ecological traits may be phylogenetically conserved, meaning that coincident changes in physiology and ecology could theoretically lead to purely incidental matches between herbivore niches and parasitoid host-use patterns (Fig. 1A) (Bailey *et al.* 2009; Desneux *et al.* 2012). However, phylogenetic information provides the possibility to identify the underlying defence mechanisms: idiosyncratic or phylogenetically conserved parasitoid absences (or clear reductions in abundance) that do not coincide with host–niche boundaries point to an effect of physiological defences (Fig. 1B), while niche-dependent parasitism can be demonstrated if distantly related insects in similar niches tend to be attacked by the same parasitoids (Fig. 1C) (Ives & Godfray 2006; Leppänen *et al.* 2013).

Here, we investigate the effects of location, host plant species, habitat and phylogenetic relationships on the composition of hymenopteran parasitoid communities that attack *Pontania* sawflies (Hymenoptera: Tenthredinidae) inducing pea- or bean-shaped galls on leaves of willows (Salicaceae: *Salix* spp.). Willow–galler–parasitoid communities are distributed across the Northern Hemisphere, and each trophic level in this system contains hundreds of species in geographically variable combinations (Argus 1997; Kopelke 2003; Roininen *et al.* 2005; Kasparyan & Kopelke 2009). To obtain a snapshot of a part of this Holarctic ‘trophic quilt’ (*sensu* Lewinsohn 2010), we DNA-barcoded parasitoid larvae collected from galls induced by seven *Pontania* species on eight *Salix* host species in subarctic and arctic–alpine habitats in three locations in northern Fennoscandia. After inferring the species composition of each community sample, we used distance- and model-based multivariate analyses and phylogenetic regression methods to test whether and how parasitoid host use is explained by geography, different galler niche dimensions and phylogenetic relationships among galler and parasitoid species. Our specific aim was to evaluate the hierarchical importance of the focal factors in relation to each other, as well as to possible physiological defences in the gallers. The results are discussed in relation to current hypotheses on top-down and bottom-up diversification effects in multitrophic food webs.

Material and methods

Parasitoid community sampling

Parasitoid communities were assessed by collecting *Pontania* galls from eight *Salix* species in Kilpisjärvi, Finland (69°03′N, 20°48′E; August 27–31, 2009), Abisko, Sweden (68°21′N, 18°49′E; August 18–22, 2009), and Tromsø, Norway (69°36′N, 19°01′E; August 25–29, 2010). These sites form the vertices of a large triangle with sides of 100–140 km; given that the distances



(A) Ecology or physiology

Host	Host niche	Parasitoid sp. 1	Parasitoid sp. 2
Host sp. 1	A	☠	
Host sp. 2	A	☠	
Host sp. 3	A	☠	
Host sp. 4	B		☠
Host sp. 5	B		☠
Host sp. 6	B		☠

(B) Physiology

Host	Host niche	Parasitoid sp. 1	Parasitoid sp. 2	Parasitoid sp. 3
Host sp. 1	A		☠	☠
Host sp. 2	A		☠	
Host sp. 3	A	☠	☠	☠
Host sp. 4	B	☠		☠
Host sp. 5	B	☠		
Host sp. 6	B	☠	☠	☠

(C) Ecology

Host	Host niche	Parasitoid sp. 1	Parasitoid sp. 2	Parasitoid sp. 3
Host sp. 1	A	☠		
Host sp. 2	A	☠		
Host sp. 3	B		☠	
Host sp. 4	A	☠		
Host sp. 5	C			☠
Host sp. 6	B		☠	

Fig. 1 Conceptual illustration of situations in which phylogenetic information on groups of specialist herbivores can be used to discern whether physiological defences or ecological traits of hosts determine parasitoid attack patterns. Niches are indicated by letters after herbivore names and colours on the phylogenetic tree, and lines crossing tree branches show directly unobservable physiological defence traits that are effective against different parasitoid species. (A) Single parasitoid species attack only (or predominantly) hosts sharing a niche or niche dimension, but groups defined by niches are reciprocally monophyletic: physiological and ecological effects cannot be separated with confidence, because the pattern could be explained by coincident shifts in host ecology and physiology. (B) Parasitoid presences and absences are erratic or partly phylogenetically conserved across the herbivore tree, but boundaries of parasitoid host ranges do not coincide with host ecology: physiological defences are the most likely explanation. (C) Distinct niches have been colonized by unrelated herbivore lineages, and herbivores sharing a niche or niche dimension also support a common parasitoid community: host ecology is likely the main factor limiting host suitability for parasitoids.

between the sites clearly exceed the typical dispersal distances of small insects (cf., e.g. van Nouhuys & Hanski 2002; Yamamura *et al.* 2003; Dulieu *et al.* 2007), for the purposes of the present study, they are considered ecological replicates. All three locations represent typical northern Lapland environments, with lowlands covered by low subarctic mountain birch (*Betula pubescens* ssp. *czerepanovii*) forests that transition into a willow-bush zone at higher elevations and, finally, to arctic–alpine habitat with ground-hugging vegetation in the highland areas.

Leaves with late-stage sawfly galls were collected from five willow species (*Salix lanata*, *Salix glauca*, *Salix lapponum*, *Salix myrsinifolia* and *Salix phylicifolia*) that grow in boreal–subarctic habitats, as well as three dwarf willow species that inhabit the arctic–alpine zone (*Salix reticulata*, *Salix polaris* and *Salix herbacea*) (Table S1 and Fig. S1, Supporting information). Each of the focal willows hosts a single species of the *Pontania viminalis* group, except for *S. polaris* and *S. herbacea*, which are galled by *Pontania aquilonis* in Kilpisjärvi and *P. aquilonis* and *P. herbaceae* in the two other locations. These two galler species induce identical galls on both hosts, but are genetically (Fig. S1, Supporting information) and morphologically distinct, and can be distinguished by their larvae (Vikberg 2003). However, identification of partly eaten larvae is uncertain, so these species are treated as one in some of the analyses below. We note that as the parasitoid communities in the *P. aquilonis* samples collected from *S. polaris* and *S. herbacea* in Kilpisjärvi are very similar to the samples from the two other localities, *P. herbaceae* evidently is either relatively rare in Abisko and Tromsø or, more likely, attacked by the same parasitoids as is *P. aquilonis* (as indicated also by the rearing-based survey of Kopelke 1999).

Altogether, 5858 galls were collected haphazardly into plastic bags and then opened in the laboratory under a dissection microscope in order to deduce the fate of their sawfly inducers (live larva/killed by parasitoid/other or unknown cause of death), and overall rates of survival and mortality were summarized across host species and locations (Table S1, Supporting information). All parasitoid larvae were placed individually in 2-mL Eppendorf tubes with 99.5% ethanol and then stored at –20°C.

Construction of the reference barcode library and community barcoding

In order to construct a reference library linking DNA barcodes to species names, a separate set of galls was collected from each site and then diverted to rearing of adult parasitoids. Galled leaves (sorted according to location and willow species) were placed in 0.5-L glass jars having a 3-cm sand layer at the bottom and a 2-cm layer of dry *Sphagnum* moss covering the sand. The rearing jars were moistened and kept at room temperature until September and then at +1°C until April, when they were returned to room temperature. At this point, the jars were covered in black plastic, and their lids were transected with a 1-cm silicone tube leading to a 30-mL plastic laboratory tube; emerging sawflies and parasitoids were attracted to the collection heads by the light entering the darkened jars via the silicone tube. The collection heads were emptied daily, and the insects were stored in 99.5% ethanol at –20°C.

Of the reared and morphologically identified parasitoid adults, 59 were used for obtaining reference barcode sequences. An additional 13 identified specimens originating from earlier rearings from a wider selection of galler species were also sequenced, in order to broaden the representation of potentially observable enemies. Genomic DNA was extracted from 1 to 2 legs or whole specimens using DNeasy Blood and Tissue Kits (Qiagen). The standard barcode portion of the mitochondrial COI gene (Hebert *et al.* 2003) was amplified using the primers hybLCO and hybHCO (Folmer *et al.* 1994; Wahlberg & Wheat 2008). The 25-μL PCRs used the reaction mix described in Leppänen *et al.* (2012) and the temperature profile of Wahlberg & Wheat (2008). PCR products were likewise purified and sequenced as described in Leppänen *et al.* (2012) and Wahlberg & Wheat (2008).

To infer parasitoid community structure in the community samples (defined by the combination of galler and willow species and location; below 'galler × willow × location') originating from the dissected galls, we DNA-barcoded 6–53 randomly selected parasitoid larvae per sample (mean $N = 25.5$), meaning that the parasitoid community on each host (defined by the combination of galler and willow species; below 'galler × willow') is estimated based on 23–89 sequences (mean $N = 70.13$) (Table S1, Supporting information). The 561 barcoded parasitoid larvae were either processed and sequenced in the same way as the reference adults, or sent for sequencing at the Canadian Centre for DNA Barcoding at the University of Guelph (www.ccdb.ca). Ichneumonid and braconid barcode sequences were of a length of 658 bp, but chalcidoid barcodes were only 652 bp long due to a two-codon deletion at alignment positions 467–472. Several chalcidoid species proved to be difficult to sequence due to the presence of a long mono-T-stretch close to the 5′ end of the barcode portion. Therefore, we designed a new forward primer, BcodeInt–f249 (5′–ATR GCW TTT CCY CGA ATA AAT AA–3′), which anneals on the 3′ side of the T-stretch, and which was then used as an internal sequencing primer or for amplifying samples that failed in the initial PCRs (resulting in a 403-bp barcode). The reference library and the final community-sample data matrix include only specimens that had barcode sequences of ≥350 bp.

Tree calculations and estimation of community structure

A neighbour-joining (NJ) tree was constructed for the reference sequences in MEGA v. 5.1 (Tamura *et al.* 2011) based on Kimura 2-parameter distances, uniform rates across sites and pairwise deletion of missing sites. Group support was assessed based on 1000 bootstrapped data sets.

In the next step, larval sequences were identified by adding them to the reference data set and calculating an NJ tree (but using only 100 bootstrap replicates due to the high number of specimens). A final NJ tree was then calculated using only larval sequences, and the number of larvae representing each parasitoid species was calculated for each community sample, *that is* all larvae originating from one galler and willow host species at a single location. These numbers were then converted into proportions by dividing the number of individuals of each parasitoid species with the total number of larvae sequenced from the same community sample.

For the purposes of the phylogeny-based community analyses, we first estimated the species-level parasitoid community for each galler (and willow host) species by summing numbers of larvae of each enemy species from all locations and then dividing the sums with the total number of parasitoid larvae sequenced for the galler species in question; for *P. aquilonis,* we used in these analyses the samples from *S. polaris* and *S. herbaceae* from Kilpisjärvi, where *P. herbaceae* does not occur, and *Pontania arcticornis* was represented by the sole location (Abisko) from which sufficient numbers of larvae were obtained. In the next step, we constructed an ultrametric phylogenetic tree for the seven focal *Pontania* species based on 2525 bp of sequence data [COI: 810 bp; *cytochrome b* (Cytb): 718 bp; *sodium–potassium adenosine triphosphatase* (NaK): 997 bp; see Malm & Nyman 2015]. Six *Euura* galler species were included in these analyses as out-groups. The data were analysed by maximum likelihood (ML) in RAxML Black-Box (Stamatakis 2006) on the CIPRES server (Miller *et al.* 2010), based on a GTR + G model of substitution, data partitioned by gene and using 100 bootstrap replicates. The resulting tree was ultrametricized using nonparametric rate smoothing (NPRS) in TREEEDIT v. 1.0 (Rambaut & Charleston 2002) and then pruned to include only *Pontania* species. A tree for the 14 parasitoid species was calculated similarly, based on the reference adult COI sequences and larval sequences for the six species that lacked reference individuals. The midpoint-rooted ML tree calculated for the full data was NPRS-ultrametricized and then pruned to include a single representative of each species. We note that although the parasitoid tree is based on barcode sequences only, its overall structure is largely congruent with the current understanding of relationships among the families and genera involved (cf. Heraty *et al.* 2011, 2013; Sharanowski *et al.* 2011; see also Quicke *et al.* 2012).

Statistical analyses

We tested the effects of host (galler × willow) and location on rates of survival and parasitism by two-way ANOVAS in IBM SPSS v. 21.0 (IBM Corporation, Somers, NY, USA). Host was included as a fixed factor because nearly all local *Pontania* species were included in the data set, and location was set as a random factor because the sampling sites are a subset of all possible locations; the interaction term was excluded from the model.

To standardize sampling effort in comparisons of enemy species richness across community samples (galler × willow × location) and hosts (galler × willow), we first constructed species-accumulation curves in different data partitions using individual-level rarefaction based on 1000 random input sequences in ESTIMATES v. 9.0.0 (Colwell 2013). The effect of host species on rarefied enemy-community richness at $N = 10$ and $N = 20$ was then tested by nonparametric Kruskal–Wallis one-way ANOVA, and the effect of habitat (when using species-level data) with a Mann–Whitney *U*-test at $N = 20$ in SPSS. *Pontania arcticornis*, which was found in sufficient numbers only in Abisko, was excluded from the sample-level analyses, and for the 2–3 samples that did not reach the rarefaction cut-offs, we used the numbers achieved at their observed sample sizes.

In order to obtain a visual representation of parasitoid community similarity across hosts and locations, we performed a nonmetric multidimensional scaling (NMDS) ordination in PC-ORD v. 5.33 (McCune & Mefford 2006), based on the proportional parasitism by each enemy species and quantitative Sørensen (=Bray–Curtis) distances among community samples. The optimal number of ordination axes and run parameters were selected using the slow-and-thorough autopilot option in PC-ORD. Multiresponse permutation procedures (MRPP) in PC-ORD were used to test the effect of location and willow species on parasitoid communities; in essence, these analyses estimate the probability of finding as small or smaller average within-group distances in ordination space under a null-hypothesis assumption of random group assignments. We also performed an MRPP test for the effect of habitat (boreal–subarctic/arctic–alpine) using a species-level data set in which each galler × willow combination was represented by the weighted mean of its parasitoid communities at the three sites (*P. aquilonis* was again represented by the samples from *S. polaris* and *S. herbaceae* from Kilpisjärvi).

Our main tests of host and location effects on parasitoid community structure were performed in a model-based framework using the MVABUND package (Wang *et al.* 2012) in R v. 3.1.2 (R Development Core Team 2014). MVABUND was used to fit a separate generalized linear model (GLM) for the abundance of each parasitoid species in each community sample, with location and host as explanatory factors and the logarithm of the number of barcoded parasitoid larvae as an offset. Statistical significance of estimated multivariate (community-level) and univariate (single species) deviances were inferred based on 999 PIT-trap resamplings of rows of the data matrix; univariate *P* values were adjusted for multiple testing. In the analysis, we employed negative binomial regression with a log-link function, after confirming that residuals did not covary systematically with fitted linear predictors. Tests for the effect of habitat on galler species-level enemy communities were performed similarly.

We evaluated the effect (=signal strength) of phylogenetic relationships among galler and parasitoid species on the species-level quantitative association matrix using Ives & Godfray's (2006) phylogenetic bipartite linear model (pblm) approach. Calculations were performed using the PICANTE v. 1.6.2 package (Kembel *et al.* 2010) in R, and implemented 1000 bootstrap replicates to estimate 95% confidence intervals for the signal arising from the galler (d_{gallers}) and parasitoid ($d_{\text{parasitoids}}$) phylogenies. Overall model fit was inferred by contrasting mean squared errors (MSE) calculated for the full model, in which d_{gallers} and $d_{\text{parasitoids}}$ are estimated from the data (MSE_{d}), with errors obtained using a model assuming no phylogenetic covariances (MSE_{star}), and a model incorporating a Brownian motion assumption of evolution (MSE_{b}). Because MSE measures error, lower values indicate a better fit of the model.

Results

Rates of survival and parasitism

Rates of survival in the 22 galler community samples ranged from 12.07% to 87.88% (mean = 54.35%) (Table S1 and Fig. S2, Supporting information). Sample-level parasitism rates were 5.15–75.86% (mean = 30.06%), and mortality by other or unknown causes 3.64–29.35% (mean = 15.58%). Because parasitism was the main cause of death in most galler samples, there was a strong negative correlation between survival and rate of parasitism (Pearson's $r = -0.924$, $R^2 = 0.854$, $P < 0.001$). According to two-way ANOVAS, survival rate was influenced by neither host species ($P = 0.059$) nor location ($P = 0.806$), and these factors also had no effect on the rate of parasitism (host, $P = 0.064$; location, $P = 0.139$).

Reference barcode library and larval identifications

The 72 sequenced reference adult parasitoids grouped into 18 distinct clusters in the NJ tree (Fig. S3, Supporting information), when clusters are defined based on a standard 2% barcode divergence limit (cf. Smith *et al.* 2011; Wirta *et al.* 2014). Morphological identifications generally agree with the sequence-based species clusters, but a mislabelling of a *Shawiana lapponica* female (PV-GG) as *Bracon* sp. was corrected based on re-examination of the voucher specimen, and two adults identified as *Pteromalus pontaniae* cluster within '*P. dolichurus*', while the latter is itself split into two groups. The smaller of these clusters consists of two individuals that were reared from galler species that do not occur in the area (*Pontania pustulator*) or that were not included in our community analyses due to their divergent gall types (*Euura mucronata*); the cluster most likely represents *Pteromalus euurae* Askew, but below it will be conservatively referred to as *Pteromalus* sp.1.

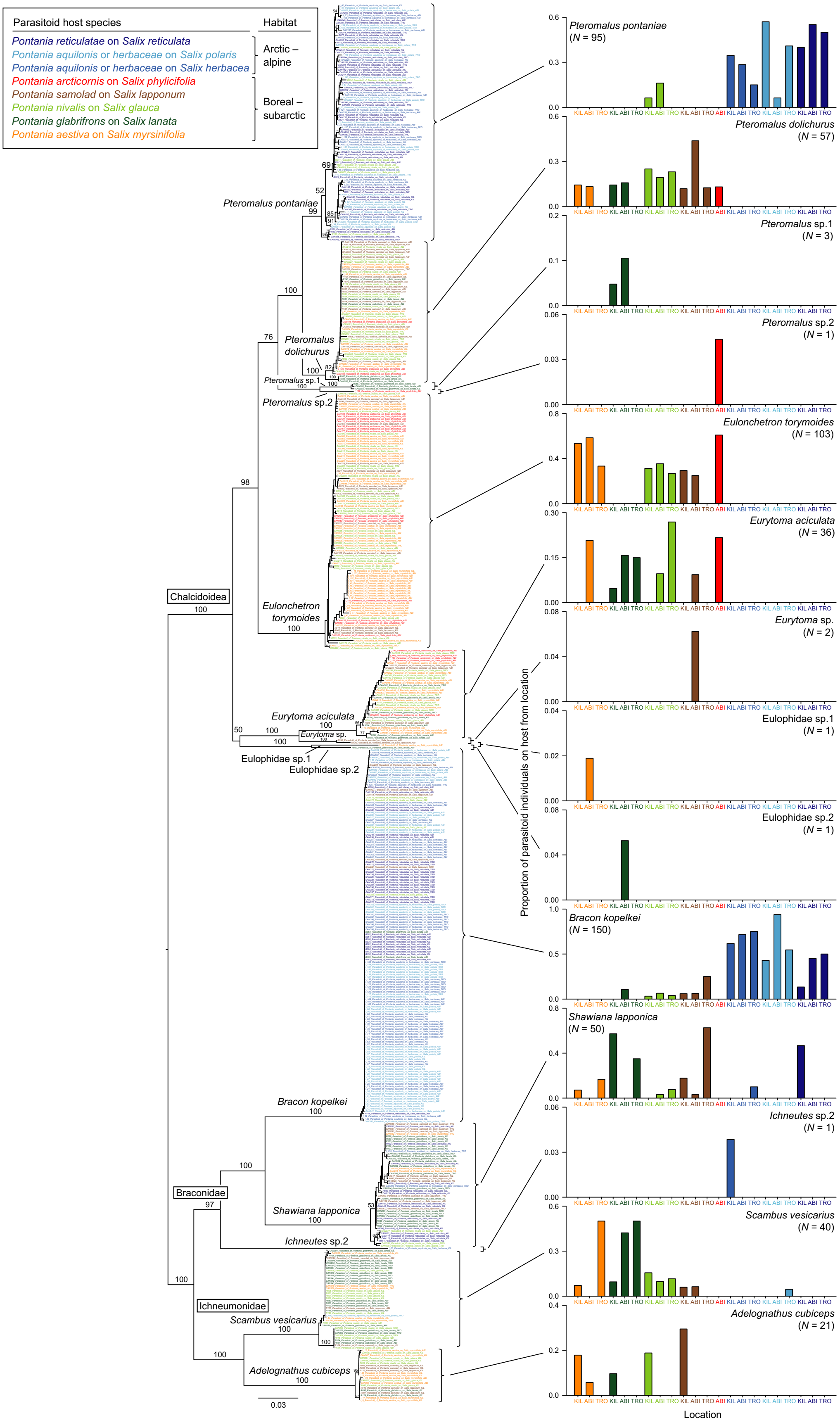
Combining the sequences of the 561 larval parasitoids with the reference data set allowed species-level identification of all but six specimens (Fig. S4, Supporting information). These unidentifiable individuals represent five putative species that were given operational familial or generic names (e.g. Eulophidae sp.1, *Eurytoma* sp.) based on their closest relatives in the reference data set and additional comparisons to sequences in GenBank and the BOLD database (www.boldsystems.org). Nine parasitoid species present in the reference data set were not found in the larval community samples, but five of these had been reared only from more southerly locations or from nonfocal sawfly species that induce different galls.

Parasitoid species richness

The composition of the parasitoid communities—calculated on the basis of the NJ tree calculated using a data set from which reference sequences had been excluded—markedly differs across the 22 community samples defined by galler and willow host species as well as locations (Fig. 2). The overall enemy community on the focal *Pontania* gallers consists of eight common species (observed total $N = 21$–150), and six species that were encountered only 1–3 times each. All of the common parasitoids are found across multiple galler (3–6) and willow (4–6) species, although their prevalence varies among hosts.

Individual-based rarefaction curves for parasitoid species richness in the community samples level off at around 10–30 specimens, indicating that our sample sizes are adequate for detecting all or nearly all enemies at most sites (Fig. S5A, Supporting information), and especially for each galler species in data sets in which communities from different sites are pooled (Fig. S5B, Supporting information). The richest enemy communities (final combined $S = 8$) are found on *Pontania samolad*, *Pontania nivalis* and *Pontania glabrifrons*, while the arctic–alpine gallers *Pontania reticulatae* and *P. aquilonis/*

Fig. 2 Neighbour-joining tree calculated for 561 parasitoid larvae on the basis of their COI barcode sequences (left), with bar charts (right) showing the proportion of individuals that belong to each parasitoid species on each galler species in each location (i.e. the sum of proportions equals 1 for each galler species in each location). Parasitoid individuals on the tree are coloured according to their galler host species and the willow host of the galler (see legend), and bars in the bar chart are coloured correspondingly. The number of individuals observed for each parasitoid species is given under species names; note that the scale on the *y*-axis differs among plots. Location codes below the bar charts are KIL = Kilpisjärvi, ABI = Abisko and TRO = Tromsø. Numbers above branches are bootstrap percentages; only values >50% are shown.


Parasitoid host species
Habitat
Pontania reticulatae on Salix reticulata
Pontania aquilonis or herbaceae on Salix polaris
Pontania aquilonis or herbaceae on Salix herbacea
Arctic – alpine
Pontania arcticornis on Salix phylicifolia
Pontania samolad on Salix lapponum
Pontania nivalis on Salix glauca
Pontania glabrifrons on Salix lanata
Pontania aestiva on Salix myrsinifolia
Boreal – subarctic
Chalcidoidea
Braconidae
Ichneumonidae
Pteromalus pontaniae
Pteromalus dolichurus
Pteromalus sp.1
Pteromalus sp.2
Eulonchetron torymoides
Eurytoma aciculata
Eurytoma sp.
Eulophidae sp.1
Eulophidae sp.2
Bracon kopelkei
Shawiana lapponica
Ichneutes sp.2
Scambus vesicarius
Adelognathus cubiceps
0.03
Pteromalus pontaniae (N = 95)
Pteromalus dolichurus (N = 57)
Pteromalus sp.1 (N = 3)
Pteromalus sp.2 (N = 1)
Eulonchetron torymoides (N = 103)
Eurytoma aciculata (N = 36)
Eurytoma sp. (N = 2)
Eulophidae sp.1 (N = 1)
Eulophidae sp.2 (N = 1)
Bracon kopelkei (N = 150)
Shawiana lapponica (N = 50)
Ichneutes sp.2 (N = 1)
Scambus vesicarius (N = 40)
Adelognathus cubiceps (N = 21)
KIL ABI TRO KIL ABI TRO KIL ABI TRO KIL ABI TRO ABI KIL ABI TRO KIL ABI TRO KIL ABI TRO
Proportion of parasitoid individuals on host from location
Location

herbaceae are attacked by fewer than four parasitoid species. Nonparametric Kruskal–Wallis one-way ANOVAS found statistically significant heterogeneity in the number of parasitoid species attacking each galler × willow combination at $N = 10$ ($P = 0.013$) as well as at $N = 20$ ($P = 0.017$). If the community samples that did not reach these rarefaction cut-offs (two and three samples, respectively) are excluded, the differences still remain statistically significant ($P = 0.021$ and $P = 0.031$, respectively). When the gallers are divided into two groups based on the habitat of their hosts, rarefied total parasitoid richness at $N = 20$ is significantly lower on arctic–alpine species than on boreal–subarctic species (Mann–Whitney *U*-test, $P = 0.036$). In this case, however, *P. aquilonis* is represented twice in the data set (on *S. polaris* and *S. herbacea*), and if the communities on these willow hosts are combined, the difference becomes only marginally significant (Mann–Whitney *U*-test, $P = 0.095$).

Determinants of parasitoid community structure

Exploratory randomization tests favoured a two-dimensional solution for NMDS ordinations both when using community samples and host species-based samples, *that is* additional axes do not result in a statistically significant improvement for the representation of the original among-sample distances. Euclidean distances among points in the final ordination based on community samples (Fig. 3A) explain 81.7% of the variation in the original quantitative Sørensen distance matrix (final stress = 13.97%). Parasitoid communities collected from the same galler and willow species are statistically significantly clustered in ordination space (MRPP test, $P < 0.0001$; this result holds even if only boreal–subarctic hosts are considered: MRPP test, $P = 0.029$), but the effect of locality is by itself nonsignificant (MRPP test, $P = 0.614$).

The model-based multivariate analysis of deviance in MVABUND likewise found a statistically significant overall effect for galler and willow host species ($\mathrm{df}_{\mathrm{res,\ diff}} = 12, 6$; Deviance = 286.20; $P = 0.001$), but not for location ($\mathrm{df}_{\mathrm{res,\ diff}} = 18, 2$; Dev = 33.34; $P = 0.137$). Results from the univariate analyses (Table S2, Supporting information) indicate that the multivariate effect is driven by host-dependent parasitism in many common enemy species (*P. pontaniae*, *P. dolichurus*, *Eulonchetron torymoides*, *Bracon kopelkei* and *Scambus vesicarius*). The effect of host species is statistically significant also when only boreal–subarctic hosts are included in the analysis ($\mathrm{df}_{\mathrm{res,\ diff}} = 6, 3$; Dev = 100.63; $P = 0.001$), but in this case also the location effect is significant ($\mathrm{df}_{\mathrm{res,\ diff}} = 9, 2$; Dev = 50.51; $P = 0.018$). The univariate deviance estimates (Table S2, Supporting information) indicate, however, that in the restricted data set, both the host and location effect may reflect patterns in one or a few parasitoid species.

The NMDS ordination based on species-level enemy communities (Fig. 3B) closely resembles the aforementioned sample-based configuration ($R^2 = 0.97$, final stress = 2.84%). A noteworthy pattern in both ordinations is the separation of the enemy communities of gallers attacking boreal–subarctic vs. arctic–alpine dwarf willows (Fig. 3B), and habitat indeed has a statistically significant effect on community structure according to both distance-based (MRPP test, $P = 0.004$) and model-based ($\mathrm{df}_{\mathrm{res,\ diff}} = 6, 1$; Dev = 86.42; $P = 0.01$) tests. If *P. aquilonis* is treated as a single species (i.e. the samples from *S. polaris* and *S. herbaceae* from Kilpisjärvi are pooled), a one-dimensional solution is favoured (not shown), but the main division still occurs along the elevation gradient, and the habitat effect remains statistically significant (MRPP test, $P = 0.011$; multivariate GLM: $\mathrm{df}_{\mathrm{res,\ diff}} = 5, 1$; Dev = 75.48; $P = 0.014$).

Phylogenetic regression on species-level parasitoid community structure (Fig. 4) indicated a very weak effect of the galler phylogeny on parasitoid–galler associations ($d_{\mathrm{gallers}} = 0.328$; 95% confidence interval = 0.006–0.670) and essentially zero signal arising from the parasitoid tree ($d_{\mathrm{parasitoids}} = 0.008$; 95% CI = 1.90×10^{-14}–0.422). The MSE term estimated for the full model with estimated *d*'s ($\mathrm{MSE_d} = 0.016$) was close to the error estimated for a model assuming no phylogenetic covariances ($\mathrm{MSE_{star}} = 0.018$), but clearly lower than values obtained when assuming Brownian motion evolution ($\mathrm{MSE_{base}} = 0.024$), again indicating lack of overall phylogenetic signal in the quantitative association matrix.

Discussion

In complex food webs formed by plants, plant-feeding insects and parasitoids, the dietary repertoire of an individual parasitoid species can consist of a single herbivore species, comprise a subset of closely related insect taxa or extend over several distantly related insect orders (Shaw 1994; Morris *et al*. 2004; Stireman 2005; Leppänen *et al.* 2013). Such wide variability in parasitoid host ranges should not, however, prevent us from attempting to identify boundaries that define distinct subcommunities or compartments within larger host–parasitoid networks, because such discontinuities can help to pinpoint key transitions in the evolution and diversification of both herbivores and parasitoids (Sugiura 2007; Cagnolo *et al*. 2011). Progress in this field has until recently been severely held back by difficulties in community sampling and species identification (Shaw 1994; Smith *et al*. 2008; Quicke 2012). A more

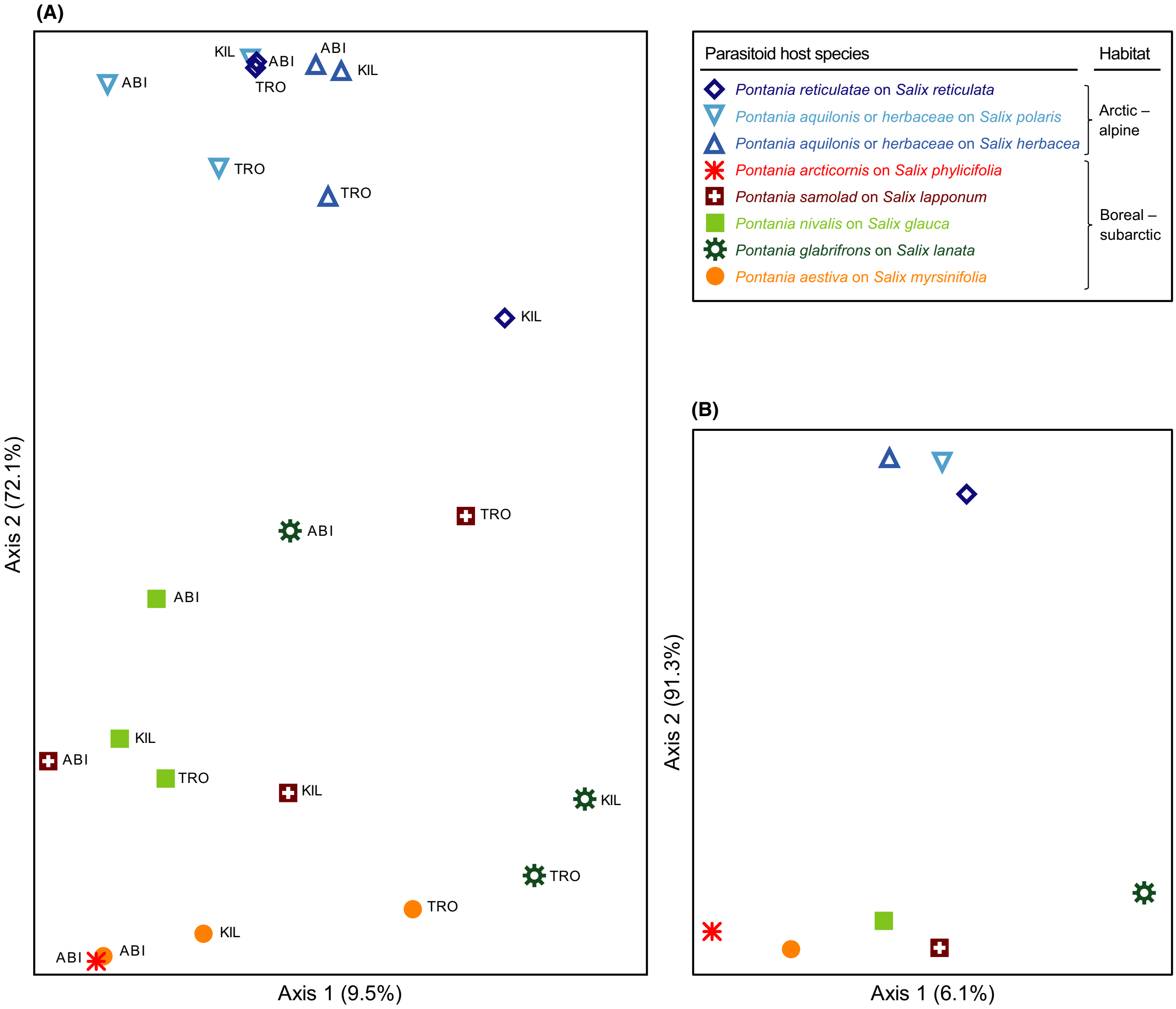
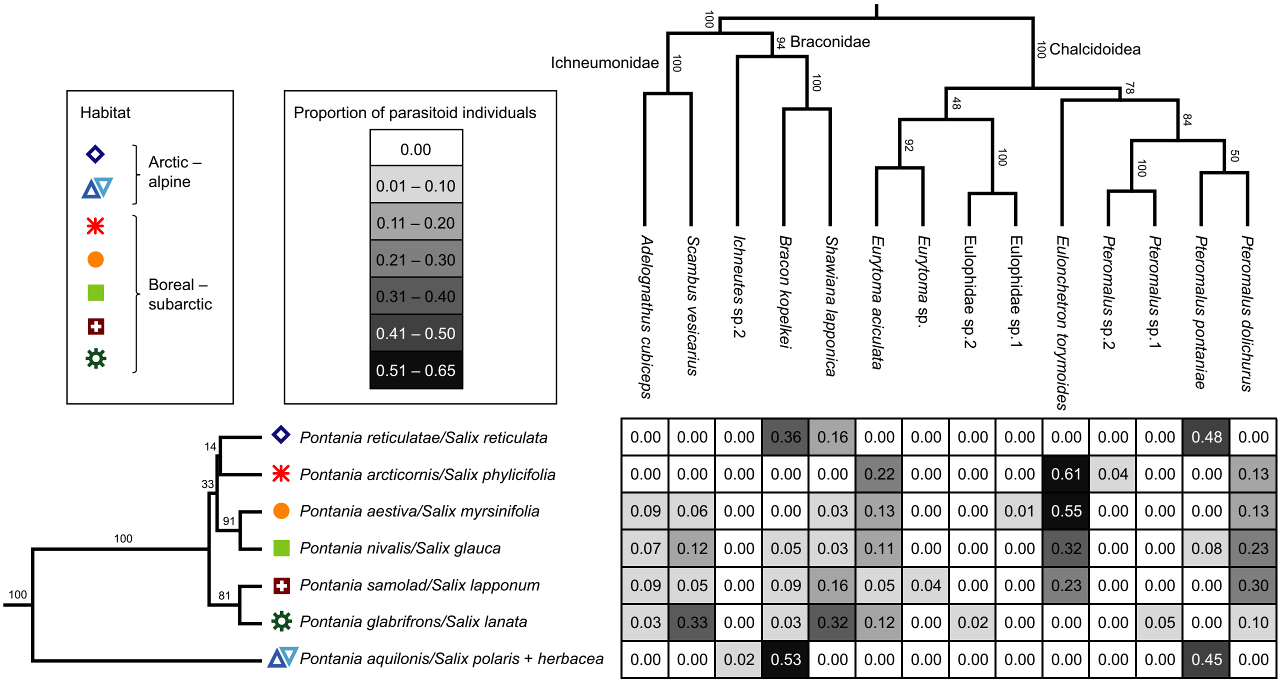


Fig. 3 (A) Two-dimensional nonmetric multidimensional scaling (NMDS) ordination plot based on quantitative Sørensen distances among parasitoid communities of seven leaf-galling *Pontania* sawfly species on eight willow species in three locations in northern Fennoscandia (KIL = Kilpisjärvi, ABI = Abisko and TRO = Tromsø). The ordination explains 81.7% of the variation in the original data, final stress = 13.97%. (B) Corresponding ordination for each galler species and willow host after combining community data from different locations (R^2 = 0.97, final stress 2.84%).

serious analytical complication can follow from lack of phylogenetic information: physiological, phenological, behavioural, morphological and ecological traits are typically phylogenetically conserved (Stireman & Singer 2003; Bailey *et al.* 2009; Desneux *et al.* 2012), so spurious correlations between host traits and parasitoid attack rates can arise unless relationships among participant species are taken into consideration (Fig. 1). The recent rise of molecular-genetic methods fortunately now allows us to, figuratively speaking, kill two birds with one stone (Quicke *et al.* 2012).

COI barcoding proved to be a fast and efficient tool for identification of parasitoid larvae within our focal willow–galler–parasitoid food web: of 561 barcoded larvae, 555 could be reliably linked to morphologically identified reference adults, and the remaining ones could be classified to operational taxa. Such direct identification of larval parasitoids circumvents problems caused by uneven mortality across parasitoid species or groups, which has been found to distort inferences in traditional rearing-based analyses (Kaartinen *et al.* 2010; Smith *et al.* 2011; Wirta *et al.* 2014). Our spatially replicated community sampling scheme, in combination with the availability of phylogenetic information of both gall inducers and parasitoids, allows for statistical tests of multiple factors that could shape the structure of



	Adelognathus cubiceps	*Scambus vesicarius*	*Ichneutes* sp.2	*Bracon kopelkei*	*Shawiana lapponica*	*Eurytoma aciculata*	*Eurytoma* sp.	Eulophidae sp.2	Eulophidae sp.1	*Eulonchetron torymoides*	*Pteromalus* sp.2	*Pteromalus* sp.1	*Pteromalus pontaniae*	*Pteromalus dolichurus*
◇ *Pontania reticulatae/Salix reticulata*	0.00	0.00	0.00	0.36	0.16	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.48	0.00
✳ *Pontania arcticornis/Salix phylicifolia*	0.00	0.00	0.00	0.00	0.00	0.22	0.00	0.00	0.00	0.61	0.04	0.00	0.00	0.13
● *Pontania aestiva/Salix myrsinifolia*	0.09	0.06	0.00	0.00	0.03	0.13	0.00	0.00	0.01	0.55	0.00	0.00	0.00	0.13
■ *Pontania nivalis/Salix glauca*	0.07	0.12	0.00	0.05	0.03	0.11	0.00	0.00	0.00	0.32	0.00	0.00	0.08	0.23
⊞ *Pontania samolad/Salix lapponum*	0.09	0.05	0.00	0.09	0.16	0.05	0.04	0.00	0.00	0.23	0.00	0.00	0.00	0.30
✲ *Pontania glabrifrons/Salix lanata*	0.03	0.33	0.00	0.03	0.32	0.12	0.00	0.02	0.00	0.00	0.00	0.05	0.00	0.10
▲▽ *Pontania aquilonis/Salix polaris + herbacea*	0.00	0.00	0.02	0.53	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.45	0.00

Fig. 4 Average proportional abundance of 14 parasitoid species on seven leaf-galling *Pontania* sawfly species, in relation to the ultrametricized galler phylogeny (to the left of the matrix) and the parasitoid phylogeny (above matrix). Numbers above branches are bootstrap percentages from the original ML phylogenetic analyses; symbols in front of galler species names denote willow host species and habitats (see legend).

parasitoid communities attacking particular galler species and populations.

Determinants of parasitoid community structure

The composition of the parasitoid community attacking any given herbivore species is typically influenced by several hierarchically organized factors (Cagnolo *et al.* 2011). The northern Fennoscandian leaf-galling *Pontania* species studied here belong to the monophyletic, species-rich tenthredinid sawfly subtribe Euurina, in which species induce leaf folds or rolls, or different types of closed galls on leaves, petioles, buds or stems of salicaceous plants (Kopelke 1999; Nyman *et al.* 2000, 2010). As in other gall-inducing insect groups (Stone & Schönrogge 2003; Bailey *et al.* 2009), the clearest transitions in parasitoid communities occur across Euurina lineages inducing galls in different parts of *Salix* hosts (Nyman *et al.* 2007). However, when only the focal *Pontania* leaf gallers are considered, our present results show that the hierarchy of determinants can be described in shorthand form as 'habitat > willow and galler host species > location > galler and parasitoid phylogeny'.

The strong and statistically significant habitat effect arises from the fact that *Pontania* gallers inhabiting treeless arctic–alpine habitats share a species-poor parasitoid community (Fig. S5, Supporting information) that is distinct from the richer community attacking gallers associated with boreal–subarctic *Salix* species (Figs 2–4). Importantly, the discontinuity is evidently based on shared ecology, because our phylogenetic regression analyses showed that neither galler nor parasitoid phylogenies explain galler–parasitoid associations (Fig. 4).

Willow and galler host species exerted a statistically significant effect on parasitoid community structure in the overall network, despite the fact that enemy communities are not strictly diagnostic for specific hosts (Fig. 3A). Comparable host plant effects on parasitism have been demonstrated using mass rearings of generalist herbivores (Lill *et al.* 2002; Murphy 2004; Farkas & Singer 2013) and phylogeny-based comparisons of specialist taxa (Ives & Godfray 2006; Nyman *et al.* 2007; Leppänen *et al.* 2013; see also Lopez-Vaamonde *et al.* 2005). The influence of willow and galler host was weaker, yet still statistically significant, within the subarctic component of the community. However, the overall host effect was in this case largely driven by a single parasitoid species, *E. torymoides*, which was completely absent from *P. glabrifrons* on *S. lanata* (Fig. 2, Table S2, Supporting information). Ascribing this absence to a plant-based effect is complicated by the fact that the comparison involves leaf galler species that are monophagous on separate willow hosts, meaning that the result could theoretically reflect a species-specific physiological or immunological defence of *P. glabrifrons* larvae. Such defences are known to be present in other plant-feeding insect groups (Schmidt *et al.* 2001; Pennacchio *et al.* 2014), and an effect of herbivore defences on parasitoid survival was recently elegantly demonstrated by Condon *et al.* (2014) in a group of closely related and ecologically near-identical *Blepharoneura* flies that infest flowers of tropical *Gurania* vines. However, in the case of *E. torymoides*, a more probable option is that the result is explained by interspecific differences in gall wall thickness or morphology (Fig. S1, Supporting information), or by differences in the phenology of gall development, all of which are composite traits influenced by both gallers and plants (cf. Craig *et al.* 1990; Stone & Schönrogge 2003). In any case, the extensive sharing of parasitoids among gallers *within* the arctic–alpine and boreal–subarctic compartments (Figs 2 and 4) points to the conclusion that host plant effects and physiological defences—if present—are generally not strong enough to lead to qualitative differences in the parasitoid communities attacking specific galler species.

Implications for vertical diversification effects

Hypotheses on vertical diversification effects in plant–herbivore–parasitoid networks revolve around the possibility that ecologically determined parasitism could facilitate colonization of novel resources and subsequent ecological speciation in herbivores (Gratton & Welter 1999; Singer & Stireman 2005), and that shifts to new niches in herbivores could induce delayed host tracking and adaptive splitting in associated parasitoid lineages (Stireman *et al.* 2006; Abrahamson & Blair 2008; Forister & Feldman 2011). Because herbivore niche shifts, changes in host–parasitoid associations and speciation events are all slow processes, direct demonstration of trophically driven diversification is impossible, and studies instead have to rely on indirect inferences. Nevertheless, demonstrations of niche-dependent parasitism at least show that conditions favouring top-down diversification exist (e.g. Murphy 2004; Farkas & Singer 2013), and the possibility of bottom-up diversification is supported by studies showing that closely related herbivores utilizing different niches are attacked by closely related yet distinct specialist parasitoids (e.g. Forbes *et al.* 2009; Kolaczan *et al.* 2009).

Genetic divergence and speciation in willow-galling sawflies is apparently largely driven by shifts among *Salix* host species (Nyman 2002; Leppänen *et al.* 2014), but the small differences in parasitoid attack rates among galler species within subarctic and arctic–alpine habitats (Fig. 2) indicates that host plant shifts are in most cases not connected with release from parasitism. On the other hand, combining our data on galler–parasitoid associations with information on the phylogenies of the participant groups strongly suggests that galler diversification has been at least partly spurred by top-down selective pressures. First, the low number of parasitoid species attacking arctic–alpine gallers is most likely explained by the fact that treeless tundra habitats have been colonized relatively recently by three galler lineages that evolved independently from boreal–subarctic ancestors (Fig. S1, Supporting information). Second, while gallers associated with dwarf willows currently share a common set of parasitoids, their original enemy communities may have been even simpler, because their shifts to tundra environments probably occurred on different continents: the closest relatives of *P. reticulatae* and *P. herbaceae* are boreal–subarctic gallers distributed in Eurasia, while the currently sympatric *P. aquilonis* is phylogenetically nested within a clade of North American species (Fig. S1, Supporting information). While overall parasitoid-inflicted mortality is often loosely related to the richness of the parasitoid community (Fig. S2, Supporting information) (Nyman *et al.* 2007; Vindstad *et al.* 2013; but see Hawkins 1994), it should be emphasized that current attack rates are unlikely to be representative of the conditions during the gallers' initial shifts to tundra habitats. Enemy-free space in herbivore–parasitoid systems is always expected to be temporary (Gratton & Welter 1999; Grosman *et al.* 2005; Heard *et al.* 2006), but even a relatively brief lag in the colonization of treeless tundra regions by the parasitoids could have provided a very strong selective 'push' for the gallers during their evolutionary transitions.

Evidence for cascading bottom-up speciation of parasitoids has previously been found in cases involving herbivore shifts among alternative host plant taxa (Stireman *et al.* 2006; Abrahamson & Blair 2008; Forbes *et al.* 2009) and changes in gall morphology (Nyman *et al.* 2007; Bailey *et al.* 2009). However, the ubiquity of diversification by delayed host tracking is still debated (Cronin & Abrahamson 2001; Althoff 2008; Lozier *et al.* 2009; Hambäck *et al.* 2013) and, according to our results, galler host shifts among willow species inhabiting the same habitat have not triggered genetic divergence or speciation in parasitoids (Fig. 2). By contrast, our finding that speciation events across the subarctic/arctic environmental divide have happened convergently within the distantly related parasitoid families Braconidae and Pteromalidae suggests that habitat shifts in herbivores can cascade to diversification in associated enemies. The species predominantly attacking arctic–alpine gallers (*P. pontaniae* and *Bracon kopelkei*) are both specialists on willow-galling sawflies (Askew 1985;

Papp 2000; Kopelke 2003), so their colonization of the tundra environment theoretically cannot predate the colonization events of the gallers. More comprehensively sampled phylogenies are required to pinpoint the sister taxa and origination times of *P. pontaniae* and *B. kopelkei*, but, judging from the short genetic distances between these species and their closest relatives on the COI barcode tree (Fig. S3, Supporting information), their sister lineages are likely to be found within parasitoid groups attacking boreal–subarctic sawfly gallers.

Conclusions and future prospects

Nearly all of the evaluated factors exerted a statistically demonstrable influence on the structure of parasitoid communities on individual *Pontania* galler species, but habitat emerged as the factor causing the largest discontinuity among enemy communities, and as the only niche boundary that appears to have induced speciation in associated parasitoids. Although the observed pattern does not directly prove that galler shifts onto tundra-inhabiting willow hosts were triggered by release from parasitism, it strongly suggests that temporary enemy-free space can be found in novel habitats, and supports the position that niche shifts in herbivores can lead to parallel ecology-based diversification in associated parasitoid lineages (Stireman *et al.* 2006; Abrahamson & Blair 2008; Forister & Feldman 2011). The most probable explanation for this lies in the stereotypical host location behaviours of parasitoids (Godfray 1994; Vinson 1998; Stilmant *et al.* 2008), as the behavioural programming and environmental preferences of species searching for victims on trees or shrubs have to be very different from the ones of species attacking hosts on ground-hugging vegetation on the open tundra. Many individual parasitoid species (Stireman & Singer 2002; Tylianakis *et al.* 2007; Stilmant *et al.* 2008) and clades (Tschopp *et al.* 2013) exhibit conservatism with respect to habitats, so we propose that analyses of parasitoid community structure and speciation across sharply demarcated biome or ecosystem boundaries could provide a very fruitful avenue for further research. In addition to insect lineages inhabiting subarctic and arctic environments along the northern tree line, comparable turnovers of enemy communities could, for example, be found in herbivore groups that span more southerly forest and grassland biomes (e.g. Peña & Wahlberg 2008; Toussaint *et al.* 2012).

The prospect of accelerating anthropogenic climate change has led to an increased interest in the structure and functioning of subarctic (Stahlhut *et al.* 2013; Vindstad *et al.* 2013) and arctic (Timms *et al.* 2013; Wirta *et al.* 2014) food webs, with the result that arctic parasitoid communities have been found to be surprisingly diverse (Fernandez-Triana *et al.* 2011; Várkonyi & Roslin 2013). These higher trophic levels are the most threatened by ongoing shrub expansion at the expense of open arctic–alpine habitats (Tape *et al.* 2006; Hallinger *et al.* 2010; Rich *et al.* 2013), as extinctions triggered by habitat loss necessarily proceed in a top-down direction (Komonen *et al.* 2000; Pearse & Altermatt 2013; Roslin *et al.* 2013). Therefore, the distinctness and high specialization of interactions found within the arctic–alpine component of our focal food web highlight the current dire situation of northern ecosystems: as shown by the species distribution models of Thuiller *et al.* (2005) and Alsos *et al.* (2009, 2012), the geographical ranges of dwarf willows and many other cold-adapted plants are expected to contract significantly in the coming decades. In our rapidly changing world, northern Fennoscandian specialized arctic–alpine plant–herbivore–parasitoid food webs are particularly vulnerable, as they are literally tucked in between the forest and the deep blue sea.

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References

Abrahamson WG, Blair CP (2008) Sequential radiation through host-race formation: herbivore diversity leads to diversity in natural enemies. In: *Specialization, Speciation, and Radiation: The Evolutionary Biology of Herbivorous Insects* (ed. Tilmon KJ), pp. 188–202. University of California Press, Berkeley, California.

Alsos IG, Alm T, Normand S, Brochmann C (2009) Past and future range shifts and loss of diversity in dwarf willow (*Salix herbacea* L.) inferred from genetics, fossils and modelling. *Global Ecology and Biogeography*, **18**, 223–239.

Alsos IG, Ehrich D, Thuiller W *et al.* (2012) Genetic consequences of climate change for northern plants. *Proceedings of the Royal Society B: Biological Sciences*, **279**, 2042–2051.

Althoff DM (2008) A test of host-associated differentiation across the “parasite continuum” in the tri-trophic interaction among yuccas, bogus yucca moths, and parasitoids. *Molecular Ecology*, **17**, 3917–3927.

Argus GW (1997) Infrageneric classification of *Salix* (Salicaceae) in the New World. *Systematic Botany Monographs*, **52**, 1–121.

Askew RR (1985) A new species of *Pteromalus* Swederus (Hymenoptera: Pteromalidae) parasitic in galls of *Pontania* Costa (Hymenoptera: Tenthredinidae). *Entomologica Scandinavica*, **16**, 233–235.

Bailey R, Schönrogge K, Cook JM *et al.* (2009) Host niches and defensive extended phenotypes structure parasitoid wasp communities. *PLoS Biology*, **7**, e1000179.

Cagnolo L, Salvo A, Valladares G (2011) Network topology: patterns and mechanisms in plant–herbivore and host–parasitoid food webs. *Journal of Animal Ecology*, **80**, 342–351.

Colwell RK (2013) *EstimateS: Statistical Estimation of Species Richness and Shared Species from Samples, version 9*. Program distributed by the author (purl.oclc.org/estimates).

Condon MA, Scheffer SJ, Lewis ML *et al.* (2014) Lethal interactions between parasites and prey increase niche diversity in a tropical community. *Science*, **343**, 1240–1244.

Craig TP, Itami JK, Price PW (1990) The window of vulnerability of a shoot-galling sawfly to attack by a parasitoid. *Ecology*, **71**, 1471–1482.

Cronin JT, Abrahamson WG (2001) Do parasitoids diversify in response to host-plant shifts by herbivorous insects? *Ecological Entomology*, **26**, 347–355.

Desneux N, Blahnik R, Delebecque CJ, Heimpel GE (2012) Host phylogeny and specialisation in parasitoids. *Ecology Letters*, **15**, 453–460.

R Development Core Team (2014) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.

Dulieu R, Merckx T, Paling N, Holloway G (2007) Using mark-release-recapture to investigate habitat use in a range of common macro-moth species. *Centre for Wildlife Assessment & Conservation E-Journal*, **1**, 1–9.

Eveleigh ES, McCann KS, McCarthy PC *et al.* (2007) Fluctuations in density of an outbreak species drive diversity cascades in food webs. *Proceedings of the National Academy of Sciences of the United States of America*, **104**, 16976–16981.

Farkas TE, Singer MS (2013) Can caterpillar density or host-plant quality explain host-plant-related parasitism of a generalist forest caterpillar assemblage? *Oecologia*, **173**, 971–983.

Fernandez-Triana J, Smith MA, Boudreault C *et al.* (2011) A poorly known high-latitude parasitoid wasp community: unexpected diversity and dramatic changes through time. *PLoS One*, **6**, e23719.

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, **3**, 294–299.

Forbes AA, Powell THQ, Stelinski LL, Smith JJ, Feder JL (2009) Sequential sympatric speciation across trophic levels. *Science*, **323**, 776–779.

Forister ML, Feldman CR (2011) Phylogenetic cascades and the origins of tropical diversity. *Biotropica*, **43**, 270–278.

Godfray HCJ (1994) *Parasitoids: Behavioral and Evolutionary Ecology*. Princeton University Press, Princeton, New Jersey.

Gratton C, Welter SC (1999) Does "enemy-free space" exist? Experimental host shifts of an herbivorous fly. *Ecology*, **80**, 773–785.

Grosman AH, van Breemen M, Holtz A *et al.* (2005) Searching behaviour of an omnivorous predator for novel and native host plants of its herbivores: a study on arthropod colonization of eucalyptus in Brazil. *Entomologia Experimentalis et Applicata*, **116**, 135–142.

Hallinger M, Manthey M, Wilmking M (2010) Establishing a missing link: warm summers and winter snow cover promote shrub expansion into alpine tundra in Scandinavia. *New Phytologist*, **186**, 890–899.

Hambäck PA, Weingartner E, Ericson L *et al.* (2013) Bayesian species delimitation reveals generalist and specialist parasitic wasps on *Galerucella* beetles (Chrysomelidae): sorting by herbivore or plant host. *BMC Evolutionary Biology*, **13**, e92.

Harvey JA, Ximénez de Embún MG, Bukovinszky T, Gols R (2012) The roles of ecological fitting, phylogeny and physiological equivalence in understanding realized and fundamental host ranges in endoparasitoid wasps. *Journal of Evolutionary Biology*, **25**, 2139–2148.

Hawkins BA (1994) *Pattern and Process in Host-Parasitoid Interactions*. Cambridge University Press, Cambridge, UK.

Heard SB, Stireman JO, Nason JD *et al.* (2006) On the elusiveness of enemy-free space: spatial, temporal, and host-plant-related variation in parasitoid attack rates on three gallmakers of goldenrods. *Oecologia*, **150**, 421–434.

Hebert PDN, Cywinska A, Ball SL, DeWaard JR (2003) Biological identifications through DNA barcodes. *Proceedings of the Royal Society B: Biological Sciences*, **270**, 313–321.

Heraty J, Ronquist F, Carpenter JM *et al.* (2011) Evolution of the hymenopteran megaradiation. *Molecular Phylogenetics and Evolution*, **60**, 73–88.

Heraty JM, Burks RA, Cruaud A *et al.* (2013) A phylogenetic analysis of the megadiverse Chalcidoidea. *Cladistics*, **29**, 466–542.

Hrcek J, Miller SE, Quicke DLJ, Smith MA (2011) Molecular detection of trophic links in a complex insect host-parasitoid food web. *Molecular Ecology Resources*, **11**, 786–794.

Hrcek J, Miller SE, Whitfield JB, Shima H, Novotny V (2013) Parasitism rate, parasitoid community composition and host specificity on exposed and semi-concealed caterpillars from a tropical rainforest. *Oecologia*, **173**, 521–532.

Ives AR, Godfray HCJ (2006) Phylogenetic analysis of trophic associations. *American Naturalist*, **168**, E1–E14.

Kaartinen R, Stone GN, Hearn J, Lohse K, Roslin T (2010) Revealing secret liaisons: DNA barcoding changes our understanding of food webs. *Ecological Entomology*, **35**, 623–638.

Kasparyan DR, Kopelke J-P (2009) Taxonomic review and key to European Ichneumon flies (Hymenoptera, Ichneumonidae), parasitoids of gall-forming sawflies of the genera *Pontania* Costa, *Phyllocolpa* Benson, and *Euura* Newman (Hymenoptera, Tenthredinidae) on willows: Part I. *Entomological Review*, **89**, 933–957.

Kembel SW, Cowan PD, Helmus MR *et al.* (2010) Picante: R tools for integrating phylogenies and ecology. *Bioinformatics*, **26**, 1463–1464.

Kitching RL (2006) Crafting the pieces of the diversity jigsaw puzzle. *Science*, **313**, 1055–1057.

Klapwijk MJ, Lewis OT (2011) Spatial ecology of multiple parasitoids of a patchily-distributed host: implications for species coexistence. *Ecological Entomology*, **36**, 212–220.

Kolaczan CR, Heard SB, Segraves KA, Althoff DM, Nason JD (2009) Spatial and genetic structure of host-associated differentiation in the parasitoid *Copidosoma gelechiae*. *Journal of Evolutionary Biology*, **22**, 1275–1283.

Komonen A, Penttilä R, Lindgren M, Hanski I (2000) Forest fragmentation truncates a food chain based on an old-growth forest bracket fungus. *Oikos*, **90**, 119–126.

Kopelke J-P (1999) Gallenerzeugende Blattwespen Europas – Taxonomische Grundlagen, Biologie und Ökologie (Tenthredinidae: Nematinae: *Euura*, *Phyllocolpa*, *Pontania*). *Courier Forschungsinstitut Senckenberg*, **212**, 1–183.

Kopelke J-P (2003) Natural enemies of gall-forming sawflies on willows (*Salix* spp.) (Hymenoptera: Tenthredinidae: *Euura*, *Phyllocolpa*, Pontania. *Entomologia Generalis*, **26**, 277–312.

Leppänen SA, Altenhofer E, Liston AD, Nyman T (2012) Phylogenetics and evolution of host-plant use in leaf-mining sawflies (Hymenoptera: Tenthredinidae: Heterarthrinae). *Molecular Phylogenetics and Evolution*, **64**, 331–341.

Leppänen SA, Altenhofer E, Liston AD, Nyman T (2013) Ecological versus phylogenetic determinants of trophic associations in a plant–leafminer–parasitoid food web. *Evolution*, **67**, 1493–1502.

Leppänen SA, Malm T, Värri K, Nyman T (2014) A comparative analysis of genetic differentiation across six shared willow host species in leaf- and bud-galling sawflies. *PLoS One*, **9**, e116286.

Lewinsohn TM (2010) A large trophic quilt. *Journal of Animal Ecology*, **79**, 1143–1145.

Lill JT, Marquis RJ, Ricklefs RE (2002) Host plants influence parasitism of forest caterpillars. *Nature*, **417**, 170–173.

Lohse K, Barton NH, Melika G, Stone GN (2012) A likelihood-based comparison of population histories in a parasitoid guild. *Molecular Ecology*, **21**, 4605–4617.

Lopez-Vaamonde C, Godfray HCJ, West SA, Hansson C, Cook JM (2005) The evolution of host use and unusual reproductive strategies in *Achrysocharoides* parasitoid wasps. *Journal of Evolutionary Biology*, **18**, 1029–1041.

Lozier JD, Roderick GK, Mills NJ (2009) Molecular markers reveal strong geographic, but not host associated, genetic differentiation in *Aphidius transcaspicus*, a parasitoid of the aphid genus *Hyalopterus*. *Bulletin of Entomological Research*, **99**, 83–96.

Malm T, Nyman T (2015) Phylogeny of the symphytan grade of Hymenoptera: new pieces into the old jigsaw(fly) puzzle. *Cladistics*, **31**, 1–17.

McCune B, Mefford MJ (2006) *PC-ORD: Multivariate Analysis of Ecological Data, ver. 5.33*. MjM Software, Gleneden Beach, Oregon, USA.

Miller MA, Pfeiffer W, Schwartz T (2010) Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: *Proceedings of the Gateway Computing Environments Workshop (GCE)*. 14 Nov. 2010, New Orleans, Louisiana.

Morris RJ, Lewis OT, Godfray HCJ (2004) Experimental evidence for apparent competition in a tropical forest food web. *Nature*, **428**, 310–313.

Murphy SM (2004) Enemy-free space maintains swallowtail butterfly host shift. *Proceedings of the National Academy of Sciences of the United States of America*, **101**, 18048–18052.

van Nouhuys S, Hanski I (2002) Colonization rates and distances of a host butterfly and two specific parasitoids in a fragmented landscape. *Journal of Animal Ecology*, **71**, 639–650.

van Nouhuys S, Hanski I (2005) Metacommunities of butterflies, their host plants, and their parasitoids. In: *Metacommunities: Spatial Dynamics and Ecological Communities* (eds Holyoak M, Leibold MA, Holt RD), pp. 99–121. University of Chicago Press, Chicago, Illinois.

Nyman T (2002) The willow bud galler *Euura mucronata* Hartig (Hymenoptera: Tenthredinidae): one polyphage or many monophages? *Heredity*, **88**, 288–295.

Nyman T, Widmer A, Roininen H (2000) Evolution of gall morphology and host-plant relationships in willow-feeding sawflies (Hymenoptera: Tenthredinidae). *Evolution*, **54**, 526–533.

Nyman T, Bokma F, Kopelke J-P (2007) Reciprocal diversification in a complex plant–herbivore–parasitoid food web. *BMC Biology*, **5**, e49.

Nyman T, Vikberg V, Smith DR, Boevé J-L (2010) How common is ecological speciation in plant-feeding insects? A "Higher" Nematinae perspective. *BMC Evolutionary Biology*, **10**, e266.

Papp J (2000) First synopsis of the species of *obscurator* species-group, genus *Bracon*, subgenus *Glabrobracon* (Hymenoptera: Braconidae, Braconinae). *Annales Historico-Naturales Musei Nationalis Hungarici*, **92**, 229–264.

Pearse IS, Altermatt F (2013) Extinction cascades partially estimate herbivore losses in a complete Lepidoptera–plant food web. *Ecology*, **94**, 1785–1794.

Peña C, Wahlberg N (2008) Prehistorical climate change increased diversification of a group of butterflies. *Biology Letters*, **4**, 274–278.

Pennacchio F, Caccia S, Digilio MC (2014) Host regulation and nutritional exploitation by parasitic wasps. *Current Opinion in Insect Science*, **6**, 74–79.

Price PW, Bouton CE, Gross P *et al.* (1980) Interactions among three trophic levels: influence of plants on interactions between insect herbivores and natural enemies. *Annual Review of Ecology and Systematics*, **11**, 41–65.

Quicke DLJ (2012) We know too little about parasitoid wasp distributions to draw any conclusions about latitudinal trends in species richness, body size and biology. *PLoS One*, **7**, e32101.

Quicke DLJ, Smith MA, Janzen DH *et al.* (2012) Utility of the DNA barcoding gene fragment for parasitic wasp phylogeny (Hymenoptera: Ichneumonoidea): data release and new measure of taxonomic congruence. *Molecular Ecology Resources*, **12**, 676–685.

Rambaut A, Charleston M (2002) *TreeEdit version 1.0 a10*. Program distributed by the authors. Department of Zoology, University of Oxford, UK.

Rich ME, Gough L, Boelman NT (2013) Arctic arthropod assemblages in habitats of differing shrub dominance. *Ecography*, **36**, 994–1003.

Roininen H, Nyman T, Zinovjev AG (2005) Biology, ecology, and evolution of gall-inducing sawflies (Hymenoptera: Tenthredinidae and Xyelidae). In: *Biology, Ecology and Evolution of Gall-inducing Arthropods, Vol. 1 and 2* (eds Raman A, Schaefer CW, Withers TM), pp. 467–494. Science Publishers Inc, Enfield, New Hampshire.

Roslin T, Várkonyi G, Koponen M, Vikberg V, Nieminen M (2013) Species-area relationships across four trophic levels – decreasing island size truncates food chains. *Ecography*, **37**, 443–453.

Schmidt O, Theopold U, Strand M (2001) Innate immunity and its evasion and supression by hymenopteran endoparasitoids. *BioEssays*, **23**, 344–351.

Schönrogge K, Crawley MJ, Walker P (1998) Invaders on the move: parasitism in the sexual galls of four alien gall wasps in Britain (Hymenoptera: Cynipidae). *Proceedings of the Royal Society B: Biological Sciences*, **265**, 1643–1650.

Sharanowski BJ, Dowling APG, Sharkey MJ (2011) Molecular phylogenetics of Braconidae (Hymenoptera: Ichneumonoidea), based on multiple nuclear genes, and implications for classification. *Systematic Entomology*, **36**, 549–572.

Shaw MR (1994) Parasitoid host ranges. In: *Parasitoid Community Ecology* (eds Hawkins BA, Sheehan W), pp. 111–144. Oxford University Press, Oxford, UK.

Singer MS, Stireman JO (2005) The tri-trophic niche concept and adaptive radiation of phytophagous insects. *Ecology Letters*, **8**, 1247–1255.

Smith MA, Rodriguez JJ, Whitfield JB *et al.* (2008) Extreme diversity of tropical parasitoid wasps exposed by iterative integration of natural history, DNA barcoding, morphology, and collections. *Proceedings of the National Academy of Sciences of the United States of America*, **105**, 12359–12364.

Smith MA, Eveleigh ES, McCann KS *et al.* (2011) Barcoding a quantified food web: crypsis, concepts, ecology and hypotheses. *PLoS One*, **6**, e14424.

Stahlhut JK, Fernández-Triana J, Adamowicz SJ *et al.* (2013) DNA barcoding reveals diversity of Hymenoptera and the dominance of parasitoids in a sub-arctic environment. *BMC Ecology*, **13**, e2.

Stamatakis A (2006) RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics*, **22**, 2688–2690.

Stilmant D, Van Bellinghen C, Hance T, Boivin G (2008) Host specialization in habitat specialists and generalists. *Oecologia*, **156**, 905–912.

Stireman JO (2005) The evolution of generalization? Parasitoid flies and the perils of inferring host range evolution from phylogenies. *Journal of Evolutionary Biology*, **18**, 325–336.

Stireman JO, Singer MS (2002) Spatial and temporal variation in the parasitoid assemblage of an exophytic polyphagous caterpillar. *Ecological Entomology*, **27**, 588–600.

Stireman JO, Singer MS (2003) Determinants of parasitoid–host associations: insights from a natural tachinid–lepidopteran community. *Ecology*, **84**, 296–310.

Stireman JO, Nason JD, Heard SB, Seehawer JM (2006) Cascading host-associated genetic differentiation in parasitoids of phytophagous insects. *Proceedings of the Royal Society B: Biological Sciences*, **273**, 523–530.

Stone GN, Schönrogge K (2003) The adaptive significance of insect gall morphology. *Trends in Ecology & Evolution*, **18**, 512–522.

Stone GN, Lohse K, Nicholls JA *et al.* (2012) Reconstructing community assembly in time and space reveals enemy escape in a Western Palearctic insect community. *Current Biology*, **22**, 532–537.

Sugiura S (2007) Structure of a herbivore–parasitoid community: are parasitoids shared by different herbivore guilds? *Basic and Applied Ecology*, **8**, 544–551.

Tamura K, Peterson D, Peterson N *et al.* (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony method. *Molecular Biology and Evolution*, **28**, 2731–2739.

Tape K, Sturm M, Racine C (2006) The evidence for shrub expansion in Northern Alaska and the Pan-Arctic. *Global Change Biology*, **12**, 686–702.

Thuiller W, Lavorel S, Araújo MB, Sykes MT, Prentice IC (2005) Climate change threats to plant diversity in Europe. *Proceedings of the National Academy of Sciences of the United States of America*, **102**, 8245–8250.

Timms LL, Bennett AMR, Buddle CM, Wheeler TA (2013) Assessing five decades of change in a high Arctic parasitoid community. *Ecography*, **36**, 1227–1235.

Toussaint EFA, Condamine FL, Kergoat GJ *et al.* (2012) Palaeoenvironmental shifts drove the adaptive radiation of a noctuid stemborer tribe (Lepidoptera, Noctuidae, Apameini) in the Miocene. *PLoS One*, **7**, e41377.

Tschopp A, Riedel M, Kropf C, Nentwig W, Klopfstein S (2013) The evolution of host associations in the parasitic wasp genus *Ichneumon* (Hymenoptera: Ichneumonidae): convergent adaptations to host pupation sites. *BMC Evolutionary Biology*, **13**, e74.

Tylianakis JM, Tscharntke T, Lewis OT (2007) Habitat modification alters the structure of tropical host-parasitoid food webs. *Nature*, **445**, 202–205.

Várkonyi G, Roslin T (2013) Freezing cold yet diverse: dissecting a high-Arctic parasitoid community associated with Lepidoptera hosts. *Canadian Entomologist*, **145**, 193–218.

Vikberg V (2003) Taxonomy of the species of *Eupontania crassipes*- and *aquilonis*-groups (Hymenoptera: Tenthredinidae: Nematinae). *Entomologica Fennica*, **14**, 129–172.

Vindstad OPL, Schott T, Hagen SB *et al.* (2013) How rapidly do invasive birch forest geometrids recruit larval parasitoids? Insights from comparison with a sympatric native geometrid. *Biological Invasions*, **15**, 1573–1589.

Vinson SB (1998) The general host selection behavior of parasitoid Hymenoptera and a comparison of initial strategies utilized by larvaphagous and oophagous species. *Biological Control*, **11**, 79–96.

Wahlberg N, Wheat CW (2008) Genomic outposts serve the phylogenomic pioneers: designing novel nuclear markers for genomic DNA extractions of lepidoptera. *Systematic Biology*, **57**, 231–242.

Wang Y, Naumann U, Wright ST, Warton DI (2012) Mvabund – an R package for model-based analysis of multivariate abundance data. *Methods in Ecology and Evolution*, **3**, 471–474.

Weiblen GD, Webb CO, Novotny V, Basset Y, Miller SE (2006) Phylogenetic dispersion of host use in a tropical insect herbivore community. *Ecology*, **87**, S62–S75.

Wirta HK, Hebert PDN, Kaartinen R *et al.* (2014) Complementary molecular information changes our perception of food web structure. *Proceedings of the National Academy of Sciences of the United States of America*, **111**, 1885–1890.

Yamamura K, Kishita M, Arakaki N, Kawamura F, Sadoyama Y (2003) Estimation of dispersal distance by mark-recapture experiments using traps: correction of bias caused by the artificial removal by traps. *Population Ecology*, **45**, 149–155.

T.N. conceived of the study; T.N. and S.A.L. planned sampling; S.A.L. collected galler survival data and larval parasitoid community samples; S.A.L., T.N. and T.E.B. reared reference parasitoid specimens; M.R.S., G.V. and V.V. identified reference adults; R.K., S.A.L. and T.N. conducted laboratory work; T.N., S.A.L. and H.R. analyzed the data; T.N. prepared figures and wrote the manuscript, with significant help from all coauthors. All authors read and approved the final manuscript.

Data accessibility

COI barcode sequences of adult parasitoid reference specimens and new sequences of the focal galler species and out-groups have been deposited in GenBank under Accession nos KT599266–KT599337 and KT599338–KT599412, respectively. These data are also available as Nexus-formatted text files in the Dryad digital repository, along with a Nexus file containing the barcode sequences of larval parasitoids, an Excel file containing the numbers of each parasitoid species found on each galler and willow species in each location, and commands and data used in the model-based community analyses and in the tests of phylogenetic effects on galler–parasitoid associations (doi: 10.5061/dryad.km75s).

Supporting information

Additional supporting information may be found in the online version of this article.

Fig. S1 Maximum-likelihood phylogeny of representative *Pontania* leaf galler species from the Holarctic region, based on 2525 bp of sequence data from the COI, Cytb and NaK genes (*Salix* host species shown after the galler names, *Euura* out-groups omitted).

Fig. S2 Rates of (A) survival and (B) parasitism in population samples of *Pontania* sawfly larvae collected from leaf galls on eight different willow species in three different locations (KIL = Kilpisjärvi, ABI = Abisko and TRO = Tromsø).

Fig. S3 Neighbour-joining tree calculated on the basis of K2P distances among COI barcode sequences of 72 reared and morphologically identified adult parasitoids.

Fig. S4 Neighbour-joining tree calculated on the basis of K2P distances among COI barcode sequences of 72 identified parasitoid adults (with names in coloured font) and 561 parasitoid larvae (in black font) collected from leaf galls induced by seven sawfly species on eight willow species in three locations in northern Fennoscandia (KIL = Kilpisjärvi, ABI = Abisko and TRO = Tromsø).

Fig. S5 Individual-based rarefaction curves for the estimated number of parasitoid species in relation to the number of sequenced parasitoid larvae.

Table S1 Sample sizes (numbers of galls), numbers and percentages of live galler larvae and larvae killed by parasitoids or other/unknown causes, and numbers of barcoded parasitoid individuals for the 22 population samples (8 galler and willow species at 1–3 locations).

Table S2 Univariate estimates of deviance and associated P values for GLM analyses of the full dataset and for boreal–subarctic host species only.