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# DNA barcoding Alaskan willow rosette gall makers (Diptera: Cecidomyiidae: *Rabdophaga*)

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## Introduction

Members of the *Rabdophaga rosaria* group form conspicuous rosette galls on a variety of willow (*Salix* spp.) hosts (Collet, 2002; Amendt, 2003) and have a holarctic distribution. Gall formation halts elongation of willow stems and alters the morphology and chemical makeup of host tissues (Gailite et al., 2005; Samsone et al., 2011). Ecologically, these flies are a keystone species for a community of insects associated with rose galls including multiple parasitoid, hyperparasitoid, and commensal species (Van Hezewijk and Roland, 2003; Collet, 2006; Skuhřavá and Thuróczy, 2007). The larvae serve as food for chickadees and tits, which pick them out of galls in winter (Van Hezewijk and Roland, 2003; Nyman et al., 2011, Figure 1). The galls themselves are avoided by moose (Kenai National Wildlife Refuge staff, 1981; Ford et al., 1995; Rea, 2012) and snowshoe hares (Ford et al., 1995).



Figure 1: A black-capped chickadee dismantling a gall induced by *Rabdophaga strobiloides* in central Michigan, December 30, 2017 (<https://flic.kr/p/232e1Yn>). Image © J. D. Sommer. Used with permission.

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The taxonomy of North American members of this group is problematic. Although Gagné (1989) provided a key and illustrations to some members of this group, the only descriptions available are the original descriptions of Osten Sacken (1862), Walsh (1864), Osten Sacken (1878), and Packard (1869), which do not enable separation of the species except through willow host species and gall morphology. Also, North American species have not been compared to Palearctic species.

Through a literature search for species that form rosette or rosette-like galls on willows we found a total of nine named *Rabdophaga* species: *Rabdophaga clavifex* (Kieffer, 1891); *Rabdophaga jaapi* Rübsaamen, 1916; *Rabdophaga rosaria* Loew, 1850; and *Rabdophaga rosariella* (Kieffer, 1897) from the Palearctic and *Rabdophaga salicisbrassicoides* Packard, 1869; *Rabdophaga saliscoryloides* Osten Sacken, 1878; *Rabdophaga salicisgnaphaloides* Osten Sacken, 1878; *Rabdophaga salicisrhodoides* Osten Sacken, 1878; and *Rabdophaga strobiloides* Osten Sacken, 1862 from the Nearctic.

We sought to determine whether one polyphagous species or multiple species of *Rabdophaga* caused rosette galls on southcentral Alaskan willow species using DNA barcodes. We also wanted to relate these entities to described *Rabdophaga* species where possible.

## Methods

Initially as a homeschool science project, the first four authors collected willow rosette galls from *Salix sitchensis* Sanson ex Bong. and *Salix barclayi* Andersson to determine whether or not these willows were galled by distinct species of *Rabdophaga*. We later expanded our sampling to a variety of willow hosts in southcentral Alaska, using the illustrations and text of Collet (2002) as a guide to these types of galls. Alaskan willow specimens that could not be identified with confidence by MLB were identified by DC. Additional specimens were obtained by TM from Kodiak Island and Michigan. A map of collection localities is given in Figure 2.

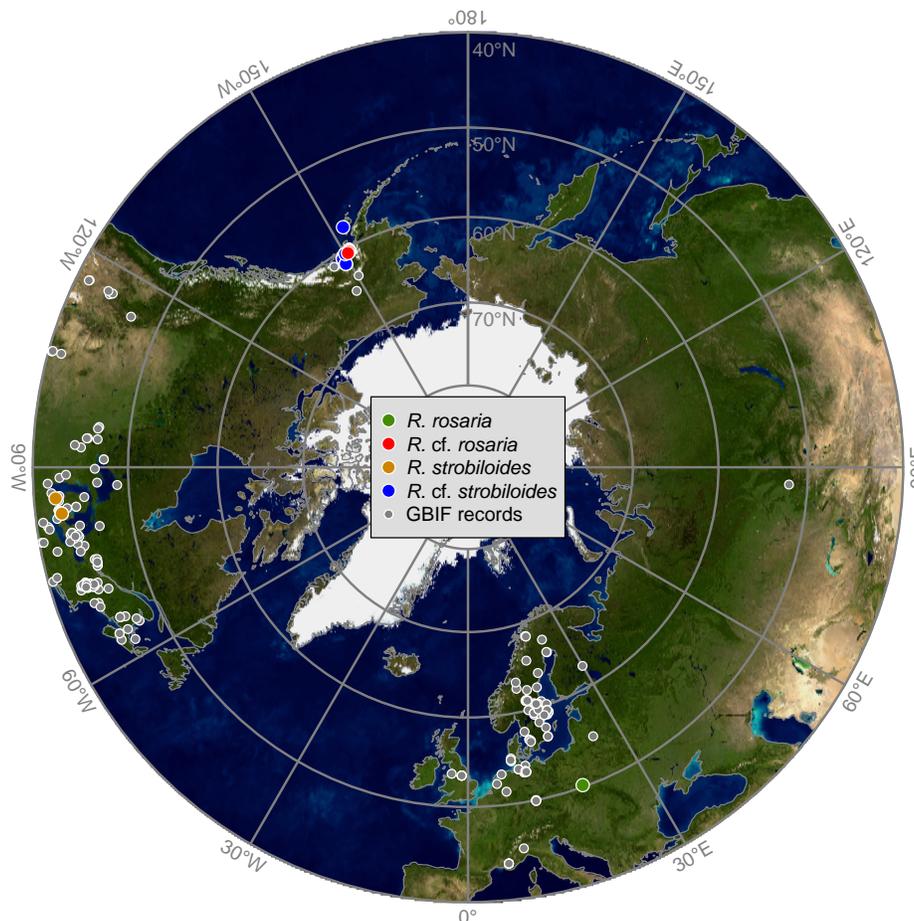


Figure 2: Polar map of collection localities color-coded as in clades in Figure 4. See the Methods section for details on the GBIF records.

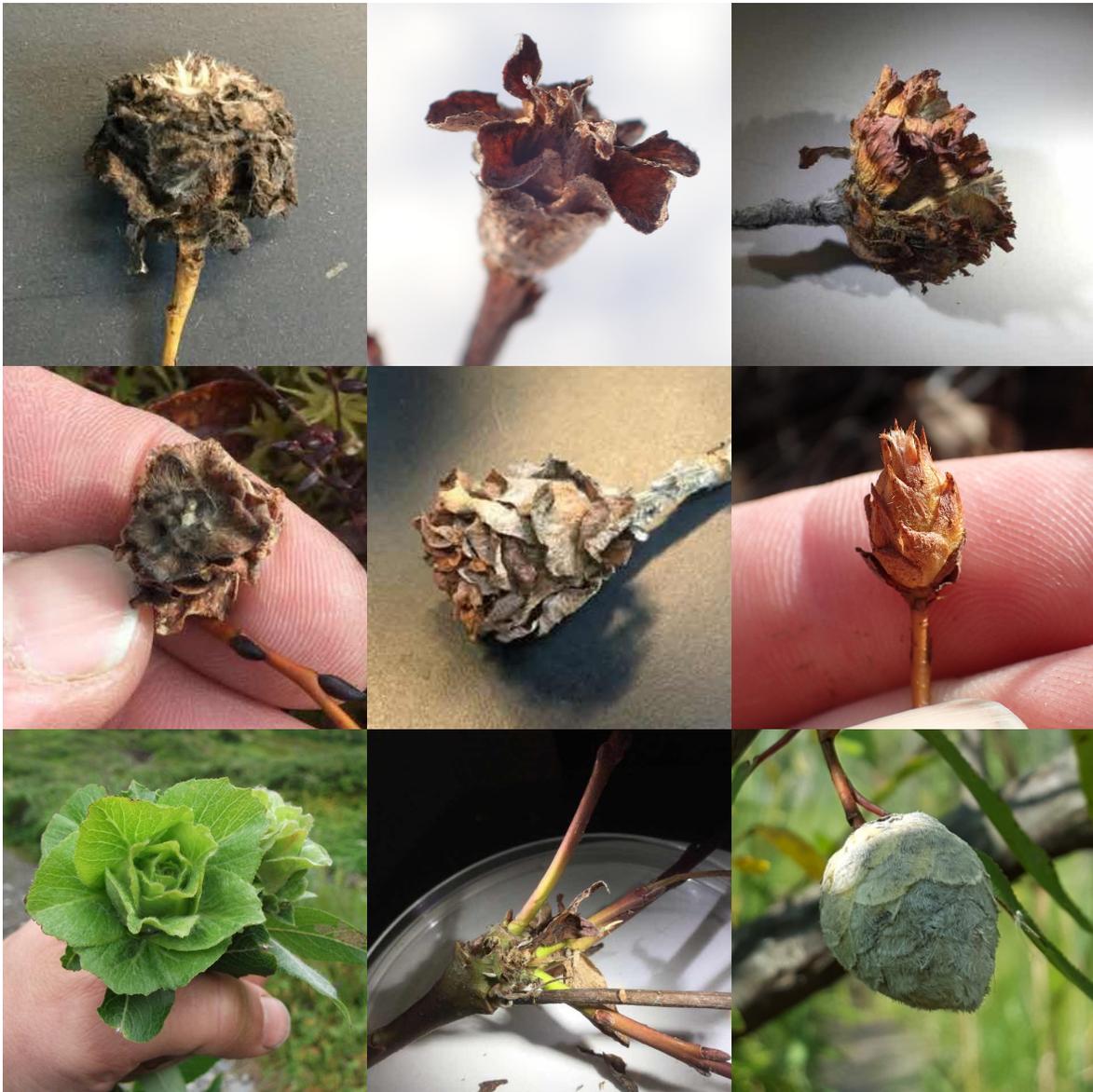


Figure 3: Representative photographs of galls sampled. **Top left:** Rosette gall on *Salix barclayi*, Alaska, Kenai Peninsula, Summit Creek, collected February 14, 2016. Arctos GUID: KNWR:Ento:11156. Image CC0 Matt Bowser (<http://arctos.database.museum/media/10510773>). **Top center:** Rosette gall on *Salix bebbiana*, Alaska, Soldotna, Ski Hill Road, February 20, 2018. Arctos GUID: KNWR:Ento:11281. Image CC0 Matt Bowser (<https://www.inaturalist.org/photos/14507792>). **Top right:** Rosette gall on *Salix commutata*, Alaska, Kenai Peninsula, Turnagain Arm, collected February 14, 2016. Arctos GUID: KNWR:Ento:11114. Image CC0 Matt Bowser (<http://arctos.database.museum/media/10522732>). **Middle left:** Rosette gall on *Salix fuscescens*, Alaska, Kenai Peninsula, Soldotna, Ski Hill Road, April 11, 2016. Arctos GUID: KNWR:Ento:11124. Image CC0 Matt Bowser (<http://arctos.database.museum/media/10508965>). **Center:** Rosette gall on *Salix hookeriana*, Alaska, Kenai Peninsula, Turnagain Arm, collected September 23, 2016. Arctos GUID: KNWR:Ento:11179. Image CC0 Matt Bowser (<http://arctos.database.museum/media/10518370>). **Middle right:** Rosette gall on *Salix pulchra*, Alaska, Kenai Peninsula, Sterling, Moose River Drive, September 26, 2016. Arctos GUID: KNWR:Ento:11169. Image CC0 Matt Bowser (<http://arctos.database.museum/media/10508962>). **Bottom left:** Rosette gall on *Salix richardsonii*, Alaska, Kenai Peninsula, Palmer Creek, August 13, 2016. Arctos GUID: KNWR:Ento:11115. Image CC BY Matt Bowser (<https://www.inaturalist.org/photos/4583073>). **Bottom center:** Rosette gall on *Salix sitchensis*, Alaska, Kenai Peninsula, Jims Landing, collected on December 7, 2015. Arctos GUID: KNWR:Ento:11097. Image CC0 Matt Bowser (<http://arctos.database.museum/media/10522737>). **Bottom right:** Willow pinecone gall on *Salix nigra*, Michigan, Kalamazoo County, Al Sabo Preserve, Portage Creek, September 16, 2017. Arctos GUID: KNWR:Ento:11280. Image CC BY-NC Tracy Melvin (<https://www.inaturalist.org/photos/13524200>).

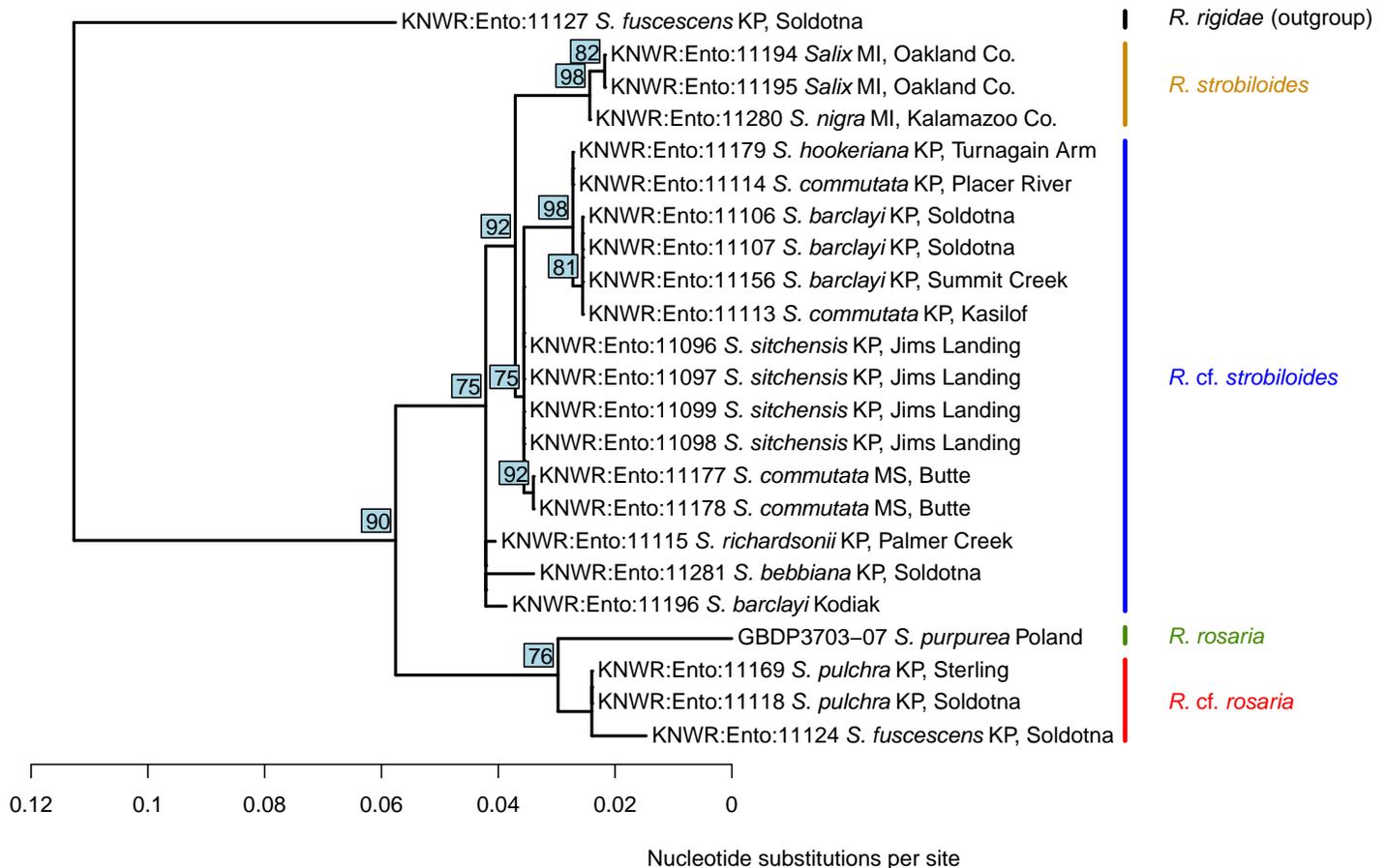


Figure 4: Phylogram of *Rabdophaga* sequences generated by PhyML. Node support values are given as percentages in blue boxes where support values were greater than 50%. KP: Kenai Peninsula, Alaska; MI: Michigan; MS: Matanuska-Susitna Borough, Alaska.

We dissected *Rabdophaga* larvae from galls and submitted whole specimens for DNA barcoding using LifeScanner kits (<http://www.lifescanner.net/>). Sequences from the DNA barcoding region of the COI gene were obtained by the Center of Biodiversity Genomics using proprietary methods. We downloaded from BOLD (Ratnasingham and Hebert, 2007) one sequence of *Rabdophaga rosaria* from Poland obtained by Sato and Yukawa (2006). We included a sequence of *Rabdophaga rigidae* (Osten Sacken, 1862) as an outgroup.

Sequences were aligned using Clustal Omega (Sievers et al., 2011) on EMBL-EBI (McWilliam et al., 2013; Li et al., 2015), accepting default parameters. A phylogenetic tree was generated from the aligned sequences using PhyML (Lefort et al., 2017) accepting default parameters.

For making a map we searched GBIF (<https://www.gbif.org/>) for all records of *Rabdophaga* on April 25, 2018. These data are available as a GBIF Occurrence Download (doi:10.15468/dl.luzr8v). For mapping we included only the nine *Rabdophaga* species of willow rosette gall mak-

ers listed above. We also added a single observation record from from Russia (<https://www.inaturalist.org/observations/7431914>).

## Results

We obtained DNA barcode sequences of rosette gall makers from the following willow hosts: *Salix barclayi*, *Salix bebbiana* Sarg., *Salix commutata* Bebb, *Salix fuscescens* Andersson, *Salix hookeriana* Barratt ex Hook., *Salix pulchra* Cham., *Salix richardsonii* Hook., and *Salix sitchensis* from southcentral Alaska; *S. barclayi* from Kodiak Island; and *Salix nigra* Marshall from Michigan (Figure 3).

Specimen data are available on Arctos (<http://arctos.database.museum>) via a saved search (doi:10.7299/X7PG1S1N). Sequence data are available on BOLD.

In the phylogenetic analysis, rosette gall makers were placed in two main clades (Figure 4) with distances be-

tween members of the clades of 0.05–0.09 nucleotide substitutions per site.

One clade included *Rabdophaga rosaria* from Poland and gallers from *S. pulchra* and *S. fuscescens* hosts with distances among members of 0.00–0.04 nucleotide substitutions per site. Distances between the Alaskan specimens and *R. rosaria* were 0.04 nucleotide substitutions per site. We tentatively identified Alaskan members of this clade as *Rabdophaga* cf. *rosaria*.

The other clade included *Rabdophaga strobiloides* from Michigan and Alaskan specimens from *S. barclayi*, *S. bebbiana*, *S. commutata*, *S. hookeriana*, and *S. richardsonii*. Distances between members of this clade were 0.00–0.03 nucleotide substitutions per site. The smallest distance between *R. strobiloides* and Alaskan members of this clade was 0.01 nucleotide substitutions per site. We tentatively identified Alaskan members of this clade as *Rabdophaga* cf. *strobiloides*.

Our sequences were organized into corresponding clades by BOLD's BIN algorithm (Ratnasingham and Hebert, 2013). Members of the clades including *R. rosaria*, *R. strobiloides*, and *R. rigidae* were placed in BINs BOLD:ADA9342 (doi:10.5883/BOLD:ADA9342), BOLD:ACZ0652 (doi:10.5883/BOLD:ACZ0652), and BOLD:ADB1445 (doi:10.5883/BOLD:ADB1445), respectively. BINs BOLD:ADA9342 and BOLD:ACZ0652 were separated by 3.75% (*p*-dist); maximum within BIN distances were 0.00% to 2.01%.

Sequences from three California specimens identified as *Rabdophaga salicisbrassicoides* have not yet been publicly released<sup>4</sup>, but the sequences are available for searching via BOLD's Identification Engine. They are 95% and 96% similar (*p*-dist) to Alaskan *R. cf. strobiloides* and *R. cf. rosaria*, respectively.

## Discussion

Both our analysis and BOLD's BIN algorithm placed Alaskan *Rabdophaga* cf. *strobiloides* with *Rabdophaga strobiloides* from Michigan. The Alaskan specimens may be conspecific with *R. strobiloides*, consistent with identifications of this species on *S. scouleriana* in British Columbia (Rea, 2012) and on *S. bebbiana* in Alberta (Van Hezewijk and Roland, 2003).

We found Alaskan members of the *R. cf. strobiloides* group induced galls on five species of willows in subgenus *Vetrix*, clade C2 of Lauron-Moreau et al. (2015). Our finding that members of this group galled *S. barclayi* and *S. commutata* corroborates the rearing experiment of Collet (2010), who found that midges reared from *S. barclayi* formed rosette galls on *S. commutata*. Elsewhere in North America, *R. strobiloides* has been recorded from *Salix cordata*

Michx., *Salix eriocephala* Michx., *Salix humilis* Marshall, *Salix nigra* Marshall (Wilson, 1968; Gagné, 1989), and *Salix scouleriana* Barratt ex Hook. (Rea, 2012), members of multiple subgenera of *Salix* in clades C1 and C2 of Lauron-Moreau et al. (2015).

Interestingly, even though *R. strobiloides* parasitizes *Salix scouleriana* in British Columbia (Rea, 2012), rosette galls on *S. scouleriana* appear to be extremely rare on the Kenai Peninsula. DC is aware of a single example of a rosette gall on *S. scouleriana* from Sterling, Alaska. MLB has not seen a rosette gall on *S. scouleriana*.

Alaskan specimens of *Rabdophaga* cf. *rosaria* were different enough from the one Old World sequence of *R. rosaria* that they may represent separate species. The Alaskan specimens, all taken from wetlands, were collected from *S. fuscescens* and *S. pulchra*, both facultative wetland species (Collet, 2002) and both members of the C2 clade of Lauron-Moreau et al. (2015). It seems more likely that the Alaskan *R. cf. rosaria* is a specialist on these willow species rather than a specialist on the wetland habitat because *R. cf. strobiloides* co-occurs with *R. cf. rosaria* on separate willow species in the same wetlands.

It remains to be resolved whether some of the genetic structure within these two groups is correlated with specialization on willow host species, geographic separation, or morphological differences. We could not conclusively answer our initial question of whether rosette makers on *S. sitchensis* and *S. barclayi* were distinct because the observed differences could have been due to geographic patterns. In the future we would like to obtain sequences from *R. cf. strobiloides* gall midges on *S. sitchensis* from other localities to help resolve this question. DC and MLB have observed that at some localities where both *S. barclayi* and *S. sitchensis* are present, rosette galls could only be found on *S. barclayi*, suggesting that the two willow species may be galled by distinct *Rabdophaga* species. Alternatively, it may be that a single species of *Rabdophaga* prefers *S. barclayi* over *S. sitchensis* where both hosts are available.

Morphologically, all galls of Alaskan *Rabdophaga* that we collected were more open than what is typical of *R. strobiloides*. In the key of Gagné (1989) they would have been identified as *R. salicisbrassicoides*, *R. salicisrhodoides*, or *Rabdophaga saliciscoryloides*, which form more open rosette galls. Most Alaskan rosette galls more closely resemble illustrations and photographs of European *R. rosaria* than any of these North American species. An exception is the unusual morphology of rosette galls on *S. sitchensis*, which often contain more than one midge larva and which often have new stems originating from leaf axils within the galls.

At present only a single sequence from this group is available from the Old World. It would be helpful to obtain DNA barcode sequences from more Palearctic rosette gall makers to clarify the relationships of these entities and

<sup>4</sup>see [http://www.boldsystems.org/index.php/Taxbrowser\\_Taxonpage?taxid=355957](http://www.boldsystems.org/index.php/Taxbrowser_Taxonpage?taxid=355957) and doi:10.7299/X73J3D8D

North American *Rabdophaga* cf. *rosaria* and *Rabdophaga* cf. *rosaria*. It would also be desirable to obtain access to sequences of North American *R. salicisbrassicoides*, *R. salicisrhodoides*, and *Rabdophaga saliciscoryloides* for comparison.

Finally, we hope that our exploratory work using DNA barcoding is followed up by a more integrated approach including morphology of the gall midges and galls.

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## How heating affects growth rate of Dubia roaches

by Fionn D. Pietsch

### Introduction

I have always loved entomology. My favorite insects are cockroaches. I currently am raising two colonies of cockroaches: one colony of Madagascar Hissing cockroaches (*Gromphadorhina portentosa*), and the other of Dubia Roaches (*Blattella germanica*). Dubia roaches live in central and south America. Madagascar hissing cockroaches live in Madagascar. Both of these areas are in the tropics and have warm climates but the Madagascar Hissing cockroaches prefer cooler habitats relative to the Dubia roaches. The only cockroaches that live in Alaska are non-native species that live in warm buildings.

You may wonder why you usually only see insects in the warmer months of the year. You may think it is because there is less food and water in the winter, which may be partially true. However, the primary reason is because of heat. Heat is one of the most important things for insect growth. I formed a hypothesis that supplementary heating

would increase the growth rate of Dubia roaches compared to their growth rate at room temperature.

### Methods

This spring, for the Interior Alaska Science Fair, I performed an experiment where I used Dubia Roaches. I had four groups which were in plastic container with air holes in them. Three of the groups sat on a 17 watt seedling heating mat (Hydrofarm Seedling Heat Mat) and the control group did not. The heated enclosures went from 23.9 °C to 26.1 °C. The unheated enclosure was 16.1 °C to 21.1 °C. All groups had the same amount of food (Nature Zones bites for Roaches) and water (Fluker's cricket quenchers calcium fortified). In each group there were about 16 juvenile cockroaches, weighing between 1.5–2 grams per group. All the groups had similar enclosures (newspaper bedding with egg crate hides).

At the beginning I weighed all of the groups. I weighed them weekly for three more weeks. At the end I calculated how much the groups grew in percentages.