

# The Phylogenetics of the Cleptoparasitic Genus *Evagetes*

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## Abstract

In this study, we furthered previous research on the taxonomy of pompilids (spider wasps) in the genus *Evagetes*. We extracted and sequenced the DNA from 18 specimens of *Evagetes* and performed phylogenetic analyses in order to answer lingering questions from the original research. Previous studies were more limited with available technology, and, therefore, no molecular studies were conducted. Using the knowledge we now have regarding DNA, and the technology available today, we were able to extract, shear, and analyze the pompilid DNA to answer three major questions. **1) Are the known species of *Evagetes* valid?** **2) How many species are in *Evagetes* (are there any unknown species)?**, and **3) What are the relationships between species in this genus?** Our study found that our understanding of *Evagetes* is just beginning. Although the described species appear valid, there are many additional species that need to be described, and the relationships of species within this group need to be re-evaluated.

## Introduction

*Evagetes* is a genus of spider wasps within the family Pompilidae. They are known as spider wasps because of their unique behavior of using spiders as a resource to get nutrients for their offspring. Spider wasps paralyze their spider prey and lay their eggs on their body. The offspring will eventually hatch from the egg and eat the host spider as they grow and mature.

The most interesting thing about *Evagetes* compared to other spider wasps lies in the fact that they are cleptoparasites. Like other spider wasps, they still rely on spiders as a host for their young. However, cleptoparasites will steal the spider of a different spider wasp, remove the egg that was placed there, and lay their own egg in its place.

Although this topic has been researched before, there is still much to learn about *Evagetes*. Different species of *Evagetes* have varying behaviors and come in a variety of shapes and sizes. Differences between the *Evagetes* species are quite subtle and would not be considered as indicating different species in other spider wasp genera. Before we can start to understand the behavior of this group of wasps, we must better understand the taxonomy.

In the past, six species within the genus *Evagetes* were identified as separate species in North America, because of minor variability in some morphological traits. These species included *Evagetes hyacinthinus*, *E. brevicornus*, *E. castaneus*, *E. scudderi*, *E. bradleyi*, and *E. texanus*. However, in the 1950's, Dr. Howard Evans decided to group them all into one; *E. hyacinthinus*. Our first task in this study was to verify his decision by reexamining these different subspecies or varieties. **The first question addressed in this study was: Are the known species of *Evagetes* valid species?** Once we understood the standing of the species within this group, we could address our second and third questions; **How many species are there in *Evagetes*?**, and **What are the relationships between the species in this genus?** Answering these three basic, yet fundamental, questions will give us a solid foundation in which we can ask more questions pertaining to behavior and its evolution within the group.

## Materials and Methods

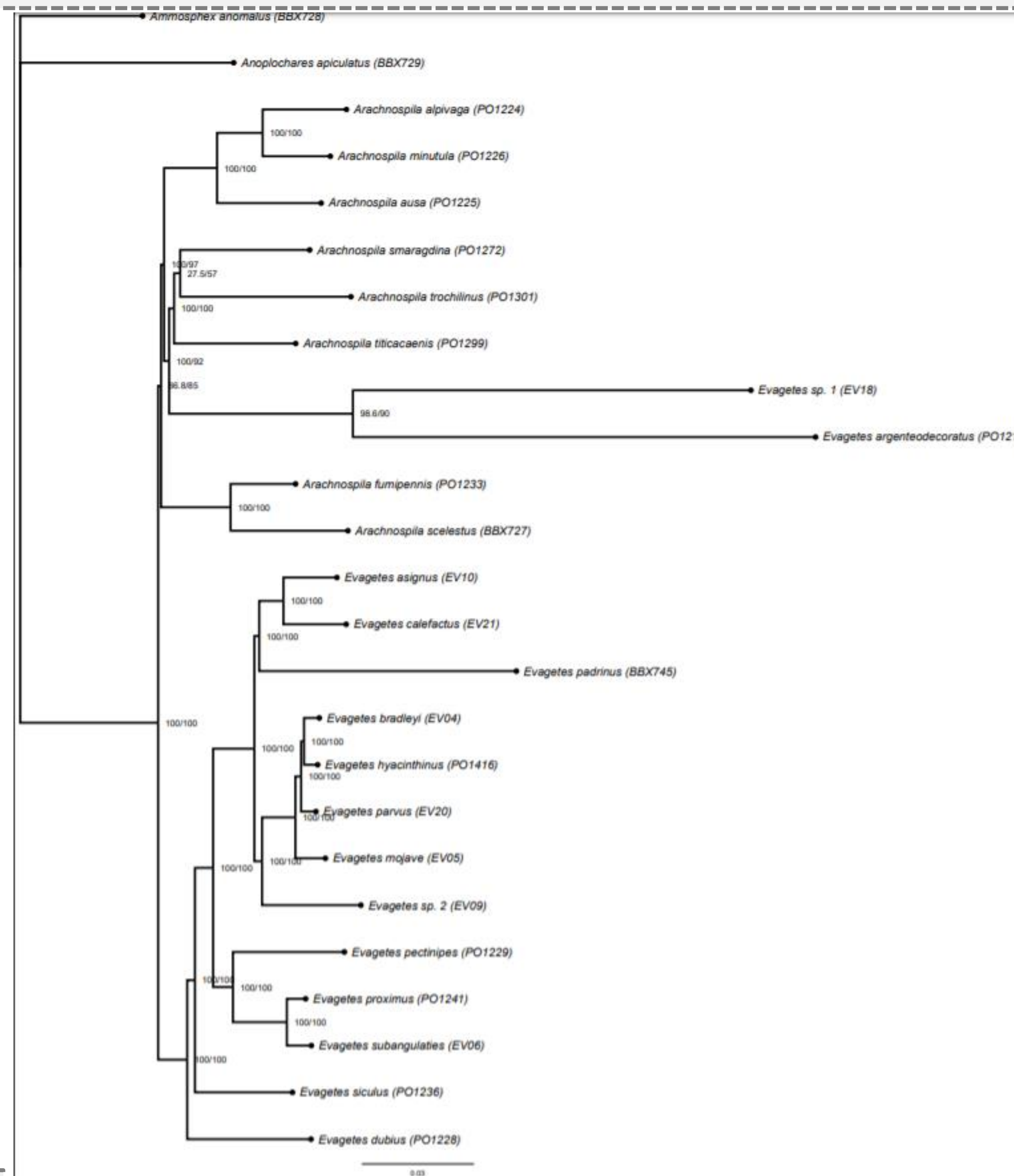
### Molecular Methods:

- DNA was extracted from the specimen using a Roche kit (Lysis Buffer, Proteinase K, and Elution, Binding, Removal, and Wash buffers).
- DNA was sheared using a Shearing Q800R2 Sonicator which is used to break DNA strands into fragments to prepare for analysis.
- DNA fragments were then collected for Library Preparation so the DNA could be sequenced.

### Phylogenetic Analyses and Methods:

- **Outgroup selection:** The three genera *AmmospheX*, *Anoplochaeres*, *Arachnospila*, served as outgroups. Morphology leads us to believe that these groups are the closest relatives to *Evagetes*.
- **Ingroup selection:** There were 13 known species of *Evagetes* included, and 2 species that were suspected to be new.

## Results



**Figure 1:** The tree shows two values of support for each node. It shows SH-like procedure approximate likelihood-ratio test (SH-aLRT) support %/ ultrafast bootstrap (UFboot) support %. The relationship shown is reliable if the SH-aLRT is  $\geq 80\%$  and UFboot  $\geq 95\%$ .



**Figure 2:** *Evagetes* sp. 1



**Figure 3:** *Evagetes* sp. 2



**Figure 4:** *Evagetes calefactus*



**Figure 5:** *Evagetes mojave*

## Discussion

### Are they all valid species?

➢ Previous attempts to create a phylogenetic tree have proven difficult. Sanger sequencing produced trees that often had significant polytomies so the relationships between species could not be fully ascertained until now. Our dataset is significantly bigger (datasets have 1,000,000 bp opposed to 1,000 bp), and we were able to see the relationships more clearly. Yes, they are all valid species, and we even determined new relationships.

### How many species are there really?

- Species in this group are difficult to identify, and the species are described based on minute differences that often wouldn't be enough to call different species in other groups.
- All the described species that we were able to sequence were in fact *valid* species according to our analyses. Interestingly, the two specimens that we suspected were new, are indeed undescribed species with interesting histories.
- *Evagetes* sp. 1 was not only a new species, but it is actually an entirely new genus closely related to *Arachnospila* and *Asthenoctenus*.
- *Evagetes* sp. 2 was thought to be *Evagetes mojave* by other taxonomists, but we have confirmed it is in fact a new species and needs to be described.

### What are the relationships between the species in this genus?

➢ *Evagetes*, as is currently described, is paraphyletic (as you can see in the tree, not all species are recovered in the same group, look at *Evagetes argentodecoratus*). The interesting thing about *E. argentodecoratus* is that Arnold (1934) placed it in the genus *Asthenoctenus*, but Evans synonymized *Asthenoctenus* with *Evagetes* in 1951. Our results suggest *Asthenoctenus* was actually valid and needs to be resurrected.

## Future Work

- There are at least 72 species of *Evagetes* that have been discovered so far. There are still countless species of wasps that have yet to be discovered, and we believe the genus *Evagetes* will continue to grow.
- This project has helped more clearly identify valid species previously described, and new species that need to be described. We even learned of a new genus. There is plenty of future work yet to be done. During this project we extracted and prepared libraries for 30 specimens, yet we were only able to include the 18 samples that were sequenced prior to widespread shutdown due to the pandemic. In the future, we would like to sequence the remaining xx samples and see how our phylogenetic tree might change.
- Once we are confident with our phylogenetic tree, and our understanding of the groups taxonomy, we can start to investigate the evolution of host use and behavior in this group.

## Citations

Evans, Howard Ensign. *Wasp Farm*. Comstock Pub. Associates, 1985.  
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