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Authors: Brown, Patricia, and Rossi, Anthony M.

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GEOGRAPHIC AND HOST-ASSOCIATED SIZE VARIATION IN THE PARASITOID WASP *TORYMUS UMBILICATUS* (HYMENOPTERA: TORYMIDAE) IN FLORIDA: IMPLICATIONS FOR HOST SURVIVAL AND COMMUNITY STRUCTURE

PATRICIA BROWN AND ANTHONY M. ROSSI*
Department of Biological Sciences, University of North Florida, Jacksonville, FL 32224, USA

*Corresponding author; E-mail: arossi@unf.edu

ABSTRACT

Acquisition of enemy-free-space has been suggested to reduce selective pressure against host range expansion in phytophagous insects. The gall midge, *Asphondylia borrichiae* Rossi and Strong (Diptera: Cecidomyiidae), which attacks the stem tips of its 3 host plants produces a spherical tumor-like growth (= gall). Juvenile stages (larvae and pupae) of *A. borrichiae* develop inside the gall; the midge spends approximately 95-98% of its life cycle embedded within the gall. During these juvenile stages, *A. borrichiae* are parasitized by 4 species of hymenopterans. Previous studies have found that one of the most common and the largest parasitoid, *Torymus umbilicatus* (Gahan), tends to dominate large galls owing to its significantly longer ovipositor, which enables it to penetrate the biggest galls and reach larvae and pupae that become unavailable to the other 3 parasitoids, which have much shorter ovipositors. Moreover, previous studies suggest that the gall midge is diverging both morphologically and genetically in sympatry. The current study is the first to provide morphological evidence that *T. umbilicatus*, which is a dominant member of the parasitoid guild that attacks *A. borrichiae*, may also be diverging in sympatry along with its host. Female *T. umbilicatus* from sea oxeye daisy (*Borrichia frutescens* [L.] DC) were significantly larger than those from alternative host plants of the gall midge, dune elder (*Iva imbricata* Walter) and marsh elder (*I. frutescens* L). Additionally, size of female *T. umbilicatus* collected from 2 geographically distant sites were significantly different and these differences were consistent with a latitudinal gradient in size between plant species. Although *T. umbilicatus* were larger from galls collected from *B. frutescens* compared to *I. frutescens* at both sites, gall diameter demonstrated a significant decline along a south-north latitudinal gradient. However, a significant interaction between plant species and site suggests that differences in *T. umbilicatus* size (and most likely their gall midge host) is caused either by phenotypic plasticity of the species at the 2 sites, or these insects (*T. umbilicatus* and gall midges) tend to be smaller with increasing latitude. Moreover, galls on *I. frutescens,* owing to their smaller size and increased crowding, decline in size at a greater rate than those from *B. frutescens* which produces significantly larger and less crowded galls.

Key Words: *Asphondylia borrichiae, Torymus umbilicatus*, gall midge, parasitoids, host-associated divergence

RESUMEN

Se ha sugerido que la adquisición de un espacio libre de enemigos puede reducir la presión selectiva en contra de la expansión del rango de hospederos de los insectos fitófagos. La mosca de la agalla, *Asphondylia borrichiae* Rossi y Strong (Diptera: Cecidomyiidae), que ataca las puntas de los tallos de sus 3 plantas hospederas produce un crecimiento como un tumor esférico (= agallas). Los estadios juveniles (larvas y pupas) de *A. borrichiae* se desarrollan dentro de las agallas, la mosquita pasa aproximadamente el 95-98% de su ciclo de vida adentro de las agallas. Durante estos estadios juveniles, *A. borrichiae* están parasitados por 4 especies himenópteros. Se han encontrado en estudios anteriores que uno de los más comunes y la más grandes parasitoides, *Torymus umbilicatus* (Gahan), tiende a dominar en las agallas grandes debido a su ovipositor significativamente más largo que le permite penetrar los más grandes agallas y llegar a las larvas y pupas que no están disponibles para los otros 3 parasitoides que tienen ovipositores mucho más cortos. Por otra parte, estudios anteriores sugieren que la mosca de la agalla esta divergiendo tanto en su morfológia y genéticamente en simpatría. El presente estudio es el primero en proveer evidencia morfológica que *T. umbilicatus*, que es un miembro dominante del grupos de parasitoides que ataca *A. borrichiae*, también puede estar divergiendo en simpatría con su hospedero. La hembra de *T. umbilicatus* recolectas sobre la margarita mar ojo de buey (*Borrichia frutescens* [L.] DC) fue significativamente mas grandes que las de las plantas hospederas alternativas de la mosca de la agalla, *Iva imbricata* Walter y *I. frutescens* L. Además, el tamaño de hembras de *T. umbilicatus* obtenida de 2 sitios geográficamente distantes fueron significativamente diferentes y estas diferencias fueron consistentes con un gradiente latitudinal en tamaño entre las especies de plantas. Aunque los individuos de *T. umbilicatus* fueron más grandes en las agallas recogidas de *B. frutescens* en comparación con *I. frutescens* en ambos sitios, el diámetro de la agalla demostró una disminución significativa a lo largo de un gradiente latitudinal de sur a norte. Sin embargo, una interacción significativa entre las especies de plantas y el sitio sugiere que las diferencias en tamaño de *T. umbilicatus* (y lo más probable su hospedero cecidómido) es causado ya sea por la plasticidad fenotípica de las especies en los 2 sitios, o estos insectos (*T. umbilicatus* y los cecidómidos) tienden a ser más pequeños con el aumento en la latitud. Por otra parte, las agallas en *I. frutescens*, debido a su menor tamaño y de esta mas apretadas sobre la hoja, se disminuyen en el tamaño a una tasa mayor que los sobre *B. frutescens* que produce agallas significativamente más grandes y menos apretadas sobre la hoja.

Palabras Clave: *Asphondylia borrichiae, Torymus umbilicatus*, mosca cecidómida, parasitoides, divergencia asociada a hospederos

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The gall midge, *Asphondylia borrichiae* (Diptera: Cecidomyiidae) Rossi and Strong, is a small ambrosia galler; females deposit eggs along with fungal conidia near the apical meristem, which results in a spherical fungal-lined gall, on its 3 host plant species (Rossi & Strong 1990; Stiling & Rossi 1997; Rossi & Stiling 1998; Rossi et al. 1999). The 3 host plants of the gall midge include sea oxeye daisy (*Borrichia frutescens* [L.] DC), marsh elder (*Iva frutescens* L.) and dune elder (*I. imbricata* Walter). All 3 plant species are closely related members of the aster family, occur in similar saline habitats (salt marshes, beaches, etc.) and they have highly sympatric distributions along the eastern and Gulf coasts of North America (USDA Plant Database Service at plants.usda.gov).

Asphondylia borrichiae galls are usually 4-chambered and each chamber contains a single larva; after eclosion larval development and pupation of the midge (and its parasitoids) occurs within the gall and adults emerge (depending upon the season) 4-8 weeks later (Rossi & Stiling 1995). Lifespan of *A. borrichiae* typically ranges from 6-12 weeks and immature midges overwinter in the galls (Rossi & Stiling 1995). Adult *Asphondylia* often live only 24-48 hours, which accounts for only 2-5% of the midge's lifecycle; most of their life (95-98%) is spent as juvenile stages embedded within the host plant's tissues (Gagné 1989; Rossi & Stiling 1995). Gall midges that develop on *B. frutescens* are significantly larger than those from either *I. frutescens* or *I. imbricata* because galls on *Borrichia* are larger and much less crowded than those from the other 2 plants (Rossi & Stiling 1995; 1998; Rossi et al. 1999; Stiling & Rossi 1994; 1998). Midges experience less competition in galls from *B. frutescens* compared to those on the 2 *Iva* species. Specifically, *A. borrichiae* that develop in sea *B. frutescens* are significantly larger than those from either *I. imbricata* or *I. frutescens*; the larger body size of midges from *B. frutescens* results in a 30-40% higher potential fitness (measured as the number of eggs at emergence) on this plant compared to the 2 *Iva* species (Rossi et al. 1999). Size- and/or host-assortative mating, coupled with different development rates of *A. borrichiae* on its host plants, may reduce gene flow between host-associated populations of the gall and promote divergence of the populations.

Four parasitoid wasps *Torymus umbilicatus* (Gahan), *Galeopsomyia haemon* (Walker), *Rileya cecidomyiae* Ashmead, and *Tenuipetiolus teredon* (Walker) parasitize juvenile stages of *Asphondylia* while they develop in the gall (Stiling et al. 1992; Stiling and Rossi 1994). Additionally, the largest and smallest species (*T. umbilicatus* and *G. haemon* respectively) are also hyperparasites that will attack the other members of the parasitoid guild as well as developing *A. borrichiae* (Stiling et al. 1992). Plant species and quality alter the relative abundance and attack rates of 4 species of parasitic wasps that parasitize the juvenile stages of the midge. For instance, the parasitoid guild that attacks the largest galls from *B. frutescens* and/or fertilized plants (which also produce larger galls) is dominated by the relatively large torymid wasp *T. umbilicatus*, while the parasitoid community of smaller galls and those on the 2 *Iva* species tend to be dominated by the smallest species of parasitoid (*Galeopsomyia haemon*). These 2 hyperparasites tend to dominate large and small galls respectively for different reasons. Specifically, *T. umbilicatus* owing to its longer ovipositor (which is more than twice as long as those from the next largest species) has the ability to oviposit last in a gall and attack juvenile stages of the midge or the other parasitoids when the galls are more mature and have a greater diameter. Conversely, the smallest species *G. haemon* is the only gregarious parasitoid in the gall community and its multiple larvae allow it to attack and overwhelm other members of the gall community including other members of the parasitoid guild and it often dominates the gall community in small galls (Stiling & Rossi 1994). Parasitism is a primary mortality factor for *A. borrichiae* and aggregate parasitism levels can reach 100% at some sites causing local extinction of the midges and it has been hypothesized that decreased size and fecundity of the midge on the 2 *Iva* spp. is partially balanced by the acquisition of enemy-free-space on these derived species (Stiling et al. 1992, 1994; Rossi & Stiling 1995; Moon & Stiling 2002; Rossi et al. 2006).

Although *A. borrichiae* is considered a single species field, laboratory and a recent genetic study provide evidence that *A. borrichiae* actually consists of distinct host-associated populations at the level of the plant genus (Rossi & Stiling 1998; Stiling & Rossi 1998; Rossi et al. 1999; Stokes et al. 2012). In particular, midges that develop in *Borrichia* are statistically larger and genetically distinct from the populations associated with the 2 species of *Iva* and much evidence suggests that the original host plant of the gall midge was sea oxeye daisy (*B. frutescens*) (Rossi & Stiling 1998; Stiling & Rossi 1994; 1998; Rossi et al. 1999). It has been hypothesized that the gall midge, probably through ovipositional mistakes, established populations on the 2 derived host plants (*I. frutescens* and *I. imbricata*) and midges from these 2 plants are significantly smaller (owing to increased larval crowding in their smaller galls) compared to midges that develop on *B. frutescens*. *Asphondylia borrichiae* exhibit consistent size and genetic differences that Stireman et al. (2006) refer to as host-associated differentiation (HAD) at the level of the plant genus. The apparent trade-off for decreased size and fecundity of *A. borrichiae* populations that utilize the 2 *Iva* spp. appears to be acquisition of enemy-free-space; midges on the derived host plants have lower parasitism rates especially during initial host plant range expansion (Stiling & Rossi 1994; Rossi & Stiling 1995; Rossi et al. 1999).

Plant-host-parasitoid systems are often tightly linked and changes in morphology, life history, genetics, etc. of the host(s) may produce a trophic cascade through the system. This study investigated whether the largest parasitoid, *Torymus umbilicatus,* exhibits HAD in size that is consistent with its gall midge host. Significant and consistent size differences suggest either phenotypic plasticity between populations of the parasitoid or a multi-trophic level cascading HAD in which both the host (*A. borrichiae*) and its primary parasitoid are diverging in sympatry at the level of the plant genus.

Materials and Methods

Collection and Preservation of Parasitoids

To examine potential host-associated size divergence in *T. umbilicatus,* galls from each of the 3 host plants within their respective overlapping ranges in 2 distant geographic and noncontiguous locations in Florida at a distance of approximately 350 km were compared. Populations used in this study were from Little Talbot Island (Duval County) located north of Jacksonville and Honeymoon Island (Pinellas County) near Tampa. Approximately 100 galls were collected from each location and species except for *I. imbricata* from Talbot Island (only 4 galls were found on this plant at this site and none of them produced any insects); therefore, this species-site combination was excluded from the study. Late-stage galls were clipped from the host plants, returned to the lab and placed in large 30-mL plastic vials where they completed development. After emergence from the galls adult *T. umbilicatus* were collected from the vials and preserved in 70% ethanol. Vials were monitored for emerging insects for at least 2-4 weeks to allow ample emergence time for all juveniles developing within the galls.

Morphometric Analysis

After emergence, female *T. umbilicatus* were collected from the vials and placed under 5X magnification on a dissecting microscope (Leica, Model MZ95, Micro Optics of Florida, Inc., Plantation, Florida 33313) fitted with an ocular micrometer to measure wing and ovipositor length. Wing length (which is typically used as a measure of size and is highly positively correlated with fecundity in many insects) and ovipositor length were measured to the nearest 0.1 ocular micrometer units (omu) (1.0 omu = 0.025 mm). Wing length was measured from the wing base (tegula) to the wing tip. Ovipositor length was measured from apex to base and, because the ovipositor is partially concealed within the abdomen, the ovipositor was pulled completely free from the abdomen with forceps prior to measuring. Pearson's correlation was used to examine the general relationship between wing and ovipositor length; because no insects were obtained from *I. imbricata* at the northern site (Little Talbot Island) and because a site or latitudinal gradient may affect insect size, only *T. umbilicatus* collected from the 3 host plants from Honeymoon Island were used in the correlation. For this same reason, a one-way ANOVA to compare ovipositor length for torymids collected from the 3 host plants used only insects from the south Florida site (Honeymoon Island) since no insects successfully developed from *I. imbricata* at the northern site. Means were compared using Tukey's HSD post-hoc test.

The relationship between plant species and site on the ovipositor length of *T. umbilicatus* were assessed using a two-way ANOVA; as mentioned above, only *B. frutescens* and *I. frutescens* could be used in this analysis since no *T. umbilicatus* were collected from *I. imbricata* at the northern site. Data were log-transformed prior to analysis to meet homogeneity of variance assumption of ANOVA (but are presented non-transformed for clarity) (SPSS, Version 10, SPSS, Inc. Chicago, Illinois 60606).

RESULTS

Wing length and ovipositor length exhibited a significant positive correlation (r = 0.576; P < 0.001; n = 61) (Fig. 1). Variation in wing length explained approximately 33% of variation in ovipositor length. *Torymus umbilicatus* tended to be larger and have longer ovipositors if they developed in galls on *B. frutescens* compared to the 2 *Iva* spp., although small sample size (especially from *I.imbricata*; n = 8) reduced the power of the statistical tests and only differences in ovipositor length approached significance ($F_{2,72}$ = 1.54; P = 0.221 and $F_{2,72}$ = 2.416 ; P = 0.097 for wing length and ovipositor length respectively, Fig. 2). [Note: if the 2 *Iva* spp. are grouped together and compared to those from *B. frutescens* the difference in ovipositor length is significant; t = 2.137; P = 0.036)].

The two-way ANOVA revealed a significant effect of host plant ($F_{1,114}$ = 23.142; P < 0.001), site ($F_{1,114}$ = 25.526; P < 0.001) as well as a significant plant × site interaction on ovipositor length ($F_{1,1}$ = 5.104; P = 0.026) (Fig. 3). Thus, while mean ovipositor length of *T. umbilicatus* is longer for parasitoids from *B. frutescens* compared to parasitoids from *I. frutescens* within a site, ovipositor lengths for both species exhibited significant differences that are consistent with a latitudinal gradient. Ovipositor lengths were much shorter at the northern site compared to the south, but the decrease in size affected parasitoids from *I. frutescens* (30% decrease) more than those from *B. frutescens* (19% decrease) resulting in a significant site × host plant interaction.

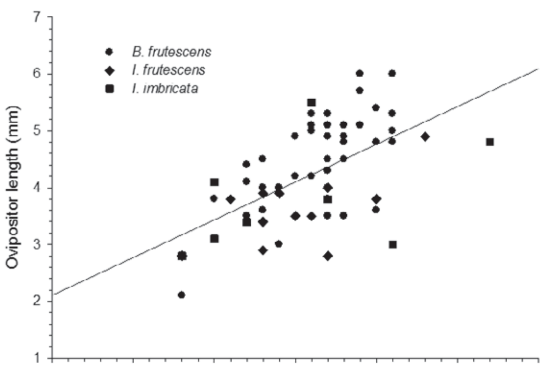

Fig. 1. Correlation between wing length (mm) and ovipositor length (mm) of *Torymus umbilicatus* from populations of its 3 host plants (*B. frutescens*, *I. frutescens* and *I. imbricata*); r = 0.576; P < 0.001).

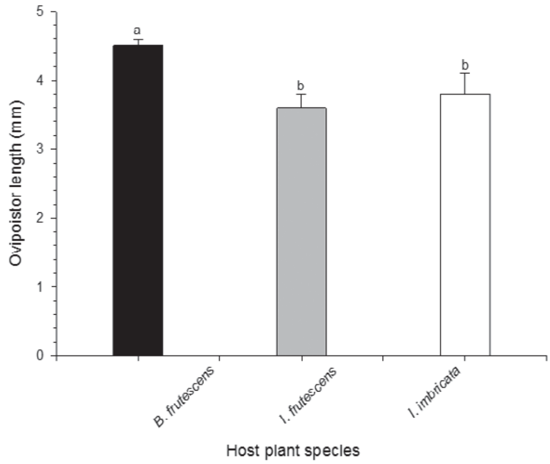

Fig. 2. Comparison of ovipositor length (mm) of *Torymus umbilicatus* from *A. borrichiae* galls from *B. frutescens*, *I. frutescens* and *I. imbricata* (Note: P = 0.097 for one-way ANOVA, but P = 0.036 for t-test in which the 2 *Iva* spp. were combined and compared to those from *B. frutescens*; see text for details). Values are mean + SE.

DISCUSSION

The current study found consistent size variation between plant-associated populations of the torymid parasitoid, *T. umbilicatus,* which attacks the juvenile stages of the gall-inducing midge *Asphondylia borrichiae*. *Torymus umbilicatus* were

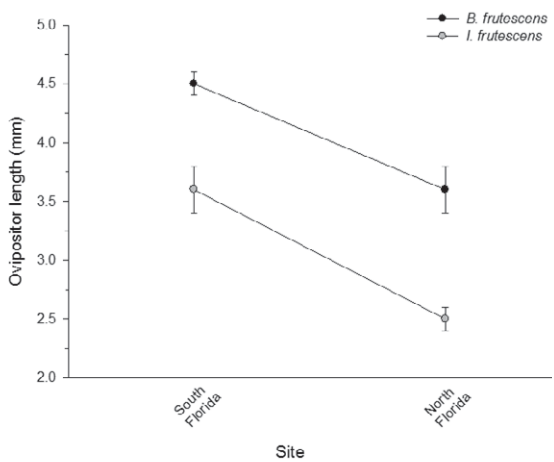

Fig. 3. Comparison of ovipositor length (mm) of *Torymus umbilicatus* from *A. borrichiae* galls collected from *B. frutescens* and *I. frutescens* from south Florida (Honeymoon Island) and north Florida (Little Talbot Island). Data were log-transformed prior to analysis, but are presented non-transformed; values are mean + SE.

consistently larger and had longer ovipositors from galls produced on *B. frutescens* compared to either species of *Iva*. These plant-associated size patterns between populations of the parasitoid are consistent with trends exhibited by the gall midge and, while some of these differences may be due to nutritional differences between the plants, much of the variability can be explained by gall size and larval crowding. Previous studies have shown that *A. borrichiae* from *B. frutescens* were significantly larger than those produced on the 2 *Iva* spp.; decreased size of the gall midge is a function of larval crowding and resource availability among the plant species (Clouse 1995; Rossi & Stiling 1995; Rossi et al. 1999). Although the average number of chambers (and hence gall midge larvae) is the same for all 3 plant species (4-5 chambers per gall), differences in gall size between the host plants results in significantly more crowded galls on the 2 *Iva* spp. compared to *B. frutescens* (Rossi & Stiling 1995; Rossi et al. 1999) Specifically, the smaller galls from *I. imbricata* and *I. frutescens* were 65% and 265% more crowded respectively compared to those from *B. frutescens* and increased larval crowding exhibited a highly negative correlation with gall midge size and potential fecundity (Rossi et al. 1999). However, the host × site study, which dropped *I. imbricata* from the analysis (since no *T. umbilicatus* from *I. imbricata* from the north site were collected), found that the effect of plant species was almost as strong as the effect of site.

The current study also suggests that either phenotypic plasticity of insect populations account for differences in their size between sites or that they are influenced by a latitudinal gradient; populations of *T. umbilicatus* from northern populations were significantly smaller compared to southern ones. However, the latitudinal effect was greater for *T. umbilicatus* from *I. frutescens* than those from *B. frutescens* which resulted in a significant plant × site interaction. Decrease in gall size at the north Florida site (Little Talbot Island) compared to the south Florida site (Honeymoon Island) is consistent with cooler temperatures at the former; the 2 sites are approximately 350 km apart and fall in different temperature zones (USDA 2012). The greater decline in size of *T. umbilicatus* that develop in *Asphondylia* galls on *Iva* spp. suggests that galls from these host plants become more crowded (and at a faster rate) compared to the much larger galls produced on *B. frutescens.* The 3 plant species have similar ecological niches and occur largely in sympatry and it has been hypothesized that the ancestral host plant for the midge was *B. frutescens* (Rossi et al. 1999; although Clouse 1995 has suggested that *I. frutescens* may have been the original host). Populations of the midge most likely became established on the 2 *Iva* spp. through ovipositional mistakes. While female midges typically oviposit on the plant from which they emerged a field study by Rossi et al. (1999) demonstrated that a small amount of "leakage" occurs between host plants. Specifically, female midges from *B. frutescens* mistakenly oviposit on the *Iva* spp. and vice versa approximately 2-3% of the time.

Interestingly, the acquisition of "enemy-free-space" has been proposed as trade-off for reduced larval performance on non-natal host plants since parasitism is initially lower after establishment on a new plant (Mira & Bernays 2002; Murphy 2004; Grosman et al. 2005). However, once the parasitoids incorporate a novel plant into their search image parasitism rates will increase (reviewed by Ishii & Shimada 2010); and, if the development times of the gall midge are asynchronous (as is the case in this system), cascading HAD of populations of both the midge and its parasitoids may develop in sympatry. Allochronic isolation between host-associated populations of the midge has already been demonstrated in field studies (Rossi & Stiling 1995, 1998, 1999; Stiling & Rossi 1998) and a recent study by Stokes et al. (2012) found genetic differences between host-associated populations of the midge at the level of the plant genus (i.e. divergence between *B. frutescens* and the 2 *Iva* spp. populations). While differences in development time between midge populations on *B. frutescens* and the 2 *Iva* spp. have already been established, galls may persist on the 3 plants throughout the year. However, *B. frutescens* is the primary host plant in the warmer months (spring-fall), while galls both *Iva* spp. are more abundant on during the colder months, (fall-spring) (Rossi & Stiling 1995). Winters in Tampa are much warmer than winters in Jacksonville and may result in faster development and increased size of galls which produces less crowded galls and larger insects. Conversely, northern gall populations on *I. frutescens* would have a longer development period owing to longer cold spells compared to the southern populations. Warmer winters in Tampa would not only provide galls on southern *I. frutescens* longer growing periods, it would also give *T. umbilicatus* populations originating from *B. frutescens* in Tampa more time to incorporate a novel host into its search image.

Incorporation of new species into a phytophagous insect's host plant range and divergence in sympatry is increasingly accepted as a primary pathway of differentiation and speciation in phytophagous insects (Craig et al. 1997; Feder 1998; Via 2000; Abrahamson et al. 2001). However, the limited number of studies investigating cascading HAD of parasitoids, have produced mixed results even within closely related systems. For instance, Stireman et al. (2006) found that parasitoids of 2 goldenrod gall makers, which have diverged genetically along host plant lines, also exhibit cascading HAD. However the eurytomid, *Eurytoma gigantea* Walsh, which attacks the stem galling tephritid fly, *Eurosta solidaginis* Fitch, another galler of the same 2 goldenrod species has not diverged in parallel with its phytophagous host (Cronin & Abrahamson 2001). In this system, differences in seasonal gall distributions, insect size and development time probably account for reduced gene flow between host-associated populations of the midge and, possibly one of its parasitoids. Prolonged isolation and differences in host quality may result in host-plant associated variation in the size and search image of the midge's parasitoids. Previous bagging studies using galls *in situ* suggest that *T. umbilicatus* becomes the dominant member of the parasitoid guild when gall diameter exceeds 7-9 mm (most likely because smaller members of the parasitoid guild are unable to reach developing insects with their shorter ovipositors) (Weis & Abrahamson 1985; Stiling & Rossi 1994; Rossi et al. 2006). Lastly, it should be mentioned that *I. imbricata*, which primarily inhabits beach dunes (rather than marshes like *B. frutescens* and *I. frutescens*) was probably the last plant colonized by the midge (and its parasitoids). Only 8 *T. umbilicatus* were collected from the *I. imbricata* at the southern site and a total of only 4 galls (but no insects) were obtained from northern populations of *I. imbricata.* This indicates that midge and *T. umbilicatus* populations in the north have yet to gain a substantial foothold on this relatively new third host plant. Future common garden and/or reciprocal transplant experiments in which parasitoids from each site and plant species are reared on their natal and alternative host plants, as well as molecular analysis of *T. umbilicatus* populations collected from galls on each plant species, should reveal whether differences in size between sites and plant species are caused by phenotypic plasticity or HAD of *Asphondylia* populations.

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