RESEARCH ARTICLE

Life history of *Parnips* and the evolutionary origin of gall wasps

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Abstract

By mechanisms that are still unknown, gall wasps (Cynipidae) induce plants to form complex galls, inside which their larvae develop. The family also includes inquilines (phytophagous forms that live inside the galls of other gall inducers) and possibly also parasitoids of gall inducers. The origin of cynipids is shrouded in mystery, but it has been clear for some time that a key group in making progress on this question is the 'figitoid inquilines'. They are gall-associated relatives of cynipids, whose biology is poorly known. Here, we report the first detailed data on the life history of a figitoid inquiline, the genus *Parnips*. Dissections of mature galls show that *Parnips nigripes* is a parasitoid of *Barbotinia oraniensis*, a cynipid that induces single-chambered galls inside the seed capsules of annual poppies (*Papaver rhoeas* and *P. dubium*). Galls with pupae of *Parnips nigripes* always contain the remains of a terminal-instar larva of *B. oraniensis*. The mandibles of the terminal-instar larva of *P. nigripes* are small and equipped with a single sharp tooth, a shape that is characteristic of carnivorous larvae. The weight of *P. nigripes* pupae closely match that of the same sex of *B. oraniensis* pupae, indicating that *Parnips* makes efficient use of its host and suggesting that ovipositing *Parnips* females lay eggs that match the sex of the host larva. Dissection of young galls show that another species of *Parnips*, hitherto undescribed, spends its late larval life as an ectoparasitoid of *Iraella hispanica*, a cynipid that induces galls in flowers of annual poppies. These and other observations suggest that *Parnips* shares the early endoparasitic-late ectoparasitic life history described for all other cynipoid parasitoids. Our findings imply that gall wasps evolved from parasitoids of gall insects. The original hosts could not have been cynipids but possibly chalcidoids, which appear to be the hosts of several extant figitoid inquilines. It is still unclear whether the gall inducers evolved rapidly from these ancestral parasitoids, or whether they were preceded by a long series of intermediate forms that were phytophagous inquilines.

Introduction

Galls are abnormal plant structures induced by foreign organisms, such as bacteria, fungi, mites or insects. They vary in complexity from simple leaf rolls to complex and well-organized structures bearing no resemblance to the attacked plant organ. The gall wasps (family Cynipidae) include some of the masters among gall inducers. Through mechanisms that are currently unknown, they induce the plant to form layers of particularly nutritive plant cells around the developing gall-wasp larva (Shorthouse 1986; Rohfritsch 1992), as well as external devices protecting the gall inhabitant from attack by natural enemies, mostly ovipositing parasitic wasps. Examples of such protective devices include secretion of adhesive substances trapping enemies, secretion of sweet substances attracting ants that deter enemies, difficult-to-pierce free-swinging or rolling larval chambers inside a larger empty space, and empty larval chambers diverting the attention of ovipositing parasitic wasps (Weld 1959, Ambrus 1974, Askew 1999b, Nicholls et al. 2017). Indeed, it seems that parasitoids are the main selective force driving the spectacular diversification of cynipid gall structure, the extended phenotype of gall wasps (Cornell 1983, Stone and Cook 1998).

The evolution of galling insects is thought to generally involve a slow transition from plant feeders without the ability to affect plant growth, through a series of intermediate forms, such as those causing simple curling of leaf margins, to true gall inducers (Price et al. 1987, Morris et al. 1999, Nyman et al. 2000). Although it has been clear for some time that the remote ancestors of gall wasps were insect-parasitic (Ronquist 1995, 1999), there has been a fair amount of speculation on the transitional stages involved in the origin of the gall inducers. Kinsey (1920) suggested that the first cynipid gall inducers evolved from stem feeders similar to some present-day cynipids associated with herbs in the Asteraceae. However, subsequent phylogenetic analyses have failed to confirm that stem feeding is the ancestral biology in the Cynipidae (Liljeblad and Ronquist 1998, Ronquist and Liljeblad 2001, Ronquist et al. 2015). Moreover, it has become clear that extant cynipid "stem feeders" actually form typical cynipid galls inside the stems or twigs they attack, it is just that the galls are not visible externally (e.g. Nieves-Aldrey et al. 2004).

Malyshev (1968) proposed a different scenario: he suggested that gall wasps evolved from seed feeders. At one point it seemed like the discovery of *Austrocynips*, reared from *Araucaria* (Araucariaceae) cones in Australia, would support Malyshev's hypothesis (Riek 1971). However, it has since been pointed out that there is no evidence that *Austrocynips* is a seed feeder, and the taxon does not appear to be as closely related to cynipids as initially thought (Ronquist 1995, 1999). There are no other seed feeders known in the Cynipoidea, which might represent surviving transitional stages supporting Malyshev's hypothesis.

A more likely scenario given the evidence we have today is that cynipids evolved from ancestors that were parasitoids inside galls induced by other insects (Ronquist 1995). This would make cynipids similar to other apocritan gall inducers, which appear to have evolved in most cases from parasitoids of hosts concealed inside plant tissues, and possibly often through intermediate forms that were parasitoids or inquilines in galls (Hanson 1995).

It has been clear for some time that a critical group in resolving the origin of gall wasps is the so-called 'figitoid inquilines', an assemblage of gall-associated relatives of cynipids. Since they were first defined as a group (Ronquist 1994), they have been divided up into five distinct subfamilies: Parnipinae, Euceroptresinae (incorrectly spelled Euceroptrinae in previous papers), Plectocynipinae, Thrasorinae and Mikeiinae (Ronquist and Nieves-Aldrey 2001, Ros-Farré and Pujade Villar 2007, Buffington 2008, Buffington and Liljeblad 2008, Buffington and Nieves-Aldrey 2011, Paretas-Martínez et al. 2011). All subfamilies are currently classified in the Figitidae, a family of insect parasitoids that form the proposed sister group of Cynipidae, where they are thought to represent early branches in the phylogeny (Ronquist 1999). This is consistent with a recent phylogenetic analysis (Ronquist et al. 2015), although the analysis leaves it open whether the figitoid inquilines are closer to core figitids or to cynipids, or whether they belong to the stem group of both (Fig. 1). It also remains unclear whether the subfamilies of figitoid inquilines all represent separate basal lineages, or whether some or all of them cluster together.

Unfortunately, little is known about the biology of the figitoid inquilines beyond the fact that they live inside galls that are apparently induced by other hymenopterans, either chalcidoids or cynipids. The Plectocynipinae have been reared from *Aditrochus* (Pteromalidae) galls on *Nothofagus* (Nothofagacae) in southern South America (Nieves-Aldrey et al. 2009, Buffington and Nieves-Aldrey 2011), but it is unclear what they do in the galls. Members of *Myrtopsen* (Thrasorinae) have been reared from galls induced by *Tanaostigmodes* (Tanaostigmatidae) on *Mimosa* (Fabacae) in South America (La Salle 1987) but there are no further details on its life history. *Mikeius hartigi* (Mikeiinae) has been reared from *Ophelimus* sp. (Eulophidae) galls on *Eucalyptus* (Myrtaceae) in Australia, but these galls host a wide range of other chalcidoids that could also be the host, if *Mikeius* is indeed a parasitoid (Buffington 2008, Paretas-Martínez et al. 2011). Several members of the Euceroptresinae are frequently reared from cynipid galls on oaks in the US, but there is no conclusive evidence that they are parasitoids and, if so, what species they attack inside the galls (Buffington and Liljeblad 2008).

Here, we present the first detailed data on the life history of a figitoid inquiline, the genus *Parnips*, constituting the subfamily Parnipinae (Ronquist and Nieves-Aldrey 2001). *Parnips* is undoubtedly the most cynipid-like of the figitoid inquilines. In fact, the species that later became the genotype (*P. nigripes*) was originally placed without comment in an existing genus of cynipid gall inducers, *Aulacidea* (Barbotin 1963). More detailed morphological study has identified at least two putative synapomorphies that *Parnips* shares with the Figitidae: the position of the Rs+M vein, issuing from the posterior end of the basal vein, and the 'ovipositor hinge', a weakness in the ninth
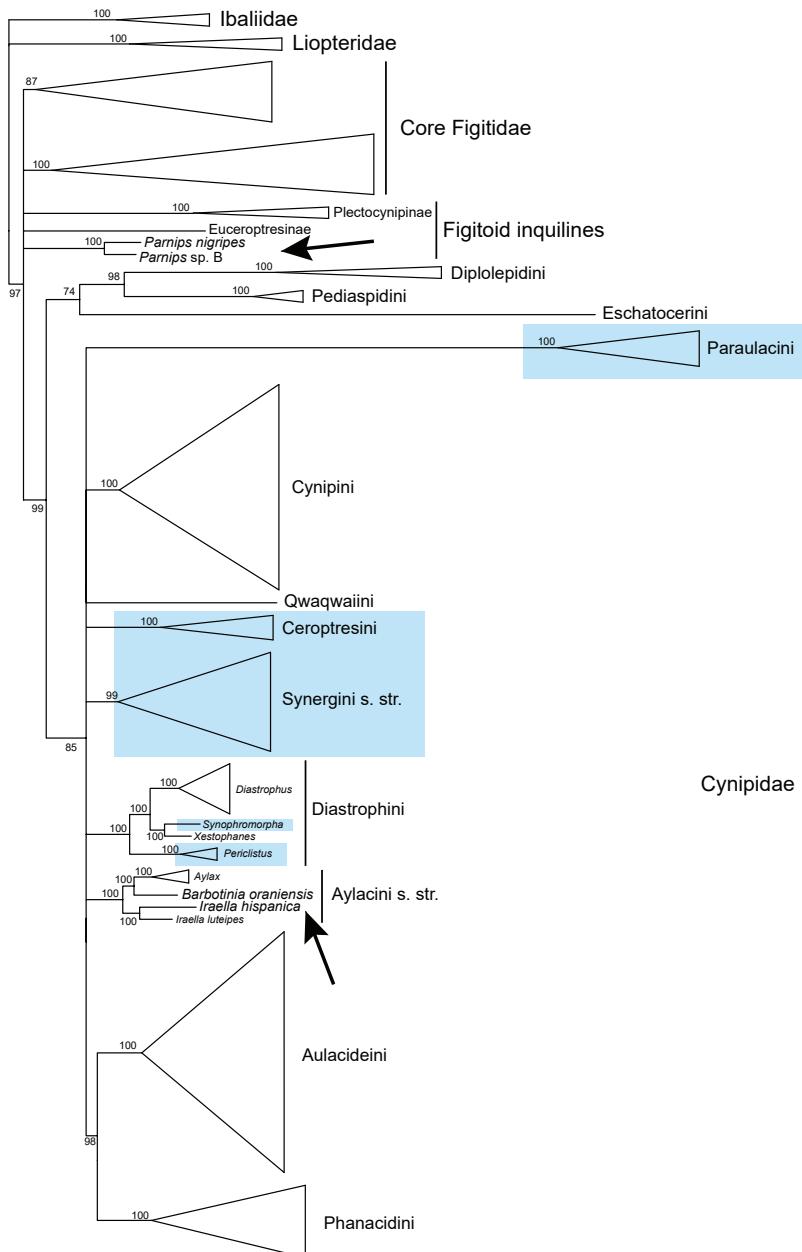
Figure 1. Phylogenetic relationships among cynipids, core figitids, figitoid inquilines and other cynipoids (simplified from Ronquist et al. 2015). Numbers are Bayesian posterior probabilities in a combined analysis of morphological and molecular data, and the width of each clade is proportional to the number of species included in the analysis. The species studied in this paper are among the figitoid inquilines and in the cynipid tribe Aylacini, and their position is shown in the tree with thick arrows. The blue boxes indicate groups that are inquilines (or parasitoids in the case of Paraulacini); all other cynipids are gall inducers as far as is known. At least two cynipid tribes appear to have originated from inquilines (Synergini and Ceroptresini), possibly also a third (Diastrophini).

tergum of the female (Ronquist 1995; Ronquist and Nieves-Aldrey 2001). A recent phylogenetic analysis based on molecular data (Buffington et al. 2007) confirms that *Parnips* does not belong to the cynipids, but it suggests that *Parnips* might be more closely related to the Cynipidae than to other figitids.

Parnips nigripes was originally reared from galls found inside seed capsules of annual poppies (*Papaver rhoeas* and *P. dubium*), collected in Algeria (Barbotin 1963). Later, *P. nigripes* was also recorded from *Iraella hispanica* galls in aborted flower galls of annual poppies collected in Northeastern Spain (Nieves-Aldrey 2005), and even more recently from similar galls collected in Romania (Pujade-Villar and Schiopu 2015). Genetic data have since indicated that the *Parnips* species associated with *Iraella* is distinct from *P. nigripes* (Ronquist et al. 2015). In the latter paper, *P. nigripes* was referred to as "*P. nigripes A*" and the new species as "*P. nigripes B*". It has turned out to be quite difficult to find morphological differences separating the two *Parnips* species, and it would be valuable to have access to more material and more genetic data to confirm the species circumscriptions. We therefore refrain from describing the new species here and simply refer to it as *Parnips* sp. B.

We have not yet been able to document the entire life cycle of any of the two species of *Parnips* but we present data here from different developmental stages bearing on the question of whether *Parnips* species are gall inducers, inquilines or parasitoids. In particular, we studied the contents of mature galls inside seed capsules of *Papaver rhoeas*, containing *Parnips nigripes* and other gall inhabitants, using various clues to infer *Parnips* life history. Did chambers containing *Parnips nigripes* larvae or pupae contain traces of other larvae that had been consumed? Was the gall smaller or larger than normal or was it differently structured when inhabited by *P. nigripes*? Did *P. nigripes* emerge before or after other gall inhabitants? We also studied whether female *P. nigripes* could be induced to oviposit in buds of the host plant in the lab, and we were able to make some observations of *Parnips* sp. B in the field. Taken together, our data clearly indicate that members of *Parnips* are parasitoids of cynipid gall inducers. Our observations are also consistent with *Parnips* having an early endoparasitic-late ectoparasitic life history, like all other cynipoid parasitoids described to date. After presenting the life-history data, we discuss the implications of these findings for our understanding of the evolutionary origin of cynipid gall inducers.

Materials and methods

Parnips nigripes. We collected a total of 151 galls of *Barbotinia oraniensis* inside seed capsules of *Papaver rhoeas* at four localities in the vicinity of Madrid, Spain – Rivas Vaciamadrid (40°19'23"N; 3°30'23"E), Arganda del Rey (40°17'9"N; 3°26'47"E), Aldea del Fresno (40°18'54"N; 4°12'28"E) and San Martín de Valdeiglesias (40°22'32"N, 4°26'50"E) – during September to October of 1997, 1998, and 1999. Of 140 intact galls without emergence holes, 46 contained healthy larvae or pupae of *Parnips nigripes* and 46 larvae or pupae of *Barbotinia oraniensis*. The remaining galls were parasitized by

at least three different species of chalcidoids, among which the rarely collected torymid *Chalcimerus borceai* was the most common.

We measured the diameter and wall thickness of the galls with a stereomicroscope fitted with an ocular micrometer. The wall thickness was difficult to measure accurately. Therefore, the measurements reported here were taken by a person who did not know about the gall contents or the purpose of the study. After dissection, the gall content was recorded and pupae of *Barbotinia oraniensis* and *Parnips nigripes* were weighed.

We stored the opened galls and their content from October until April or May at 8–10 C in small glass vials and then transferred them to room temperature. We kept emerging specimens of *Barbotinia* and *Parnips* in separate cages with free access to sucrose solution and water. Individual females of *Barbotinia* or *Parnips* were then transferred to separate cages where they were offered young *Papaver rhoeas* plants for oviposition.

***Parnips* sp. B**. Galls of *Iraella hispanica* in flowers of *Papaver rhoeas* were collected at three localities in Northeastern Spain – Marça, Tarragona (41°07'20"N, 0°48'38"E); Gandesa, Tarragona (41°2'43"N; 0°27'9"E), and between Caspe and Bujaraloz, Zaragoza (41°20'7"N; 0°5'16"E) – in May of 2002 (at Marça) and 2003 (at all localities). Some galls were opened immediately, others were reared in the lab.

Results

Parnips nigripes. Adults of *P. nigripes* are similar in general habitus to the adults of the host gall inducer, *Barbotinia oraniensis* (Fig. 2), but detailed study reveals numerous differences separating the two species. The galls of *B. oraniensis* are single-chambered and occur inside seed capsules (Fig. 3). Usually there are 1–3 galls inside each capsule, but occasionally larger numbers occur. The dissections showed that galls with healthy *Barbotinia* larvae or pupae never contained traces of foreign insects (Fig. 4a), whereas galls occupied by *Parnips* larvae or pupae always contained remnants of a host larva (arrow, Fig. 4b). The host remains were minute, only consisting of cuticular fragments and mandibles. The mandibles were dissected from 15 of the host remains and were identified in all cases as the mandibles of the terminal-instar larva of *B. oraniensis* (Fig. 5a). The larval mandibles of the other gall inhabitants - the chalcidoids and *P. nigripes* (Fig. 5b) - were quite different from the mandibles of *B. oraniensis*, allowing easy identification. The mandibles of the terminal-instar larva of *Parnips* (Fig. 5b) are small and delicate and have a single, long, pointed incisor with a weak secondary tooth along its upper margin. The mandibles of the terminal-instar larva of *Barbotinia* have much larger and stronger mandibles with two to three blunt teeth of almost equal size (Fig. 5a).

Galls containing male pupae were significantly smaller than galls containing female pupae, both for *Barbotinia* and *Parnips* (Fig. 6a). However, there was no difference between the size of the *Barbotinia* and *Parnips* galls, after the sexes had been paired. Similarly, female pupae were significantly heavier than male pupae in both species, but *Parnips* pupae were comparable in weight to *Barbotinia* pupae (Fig. 6b).

Figure 2. Habitus of the adult female of *Barbotinia oraniensis* (**a**) and its parasitoid *Parnips nigripes* (**b**).
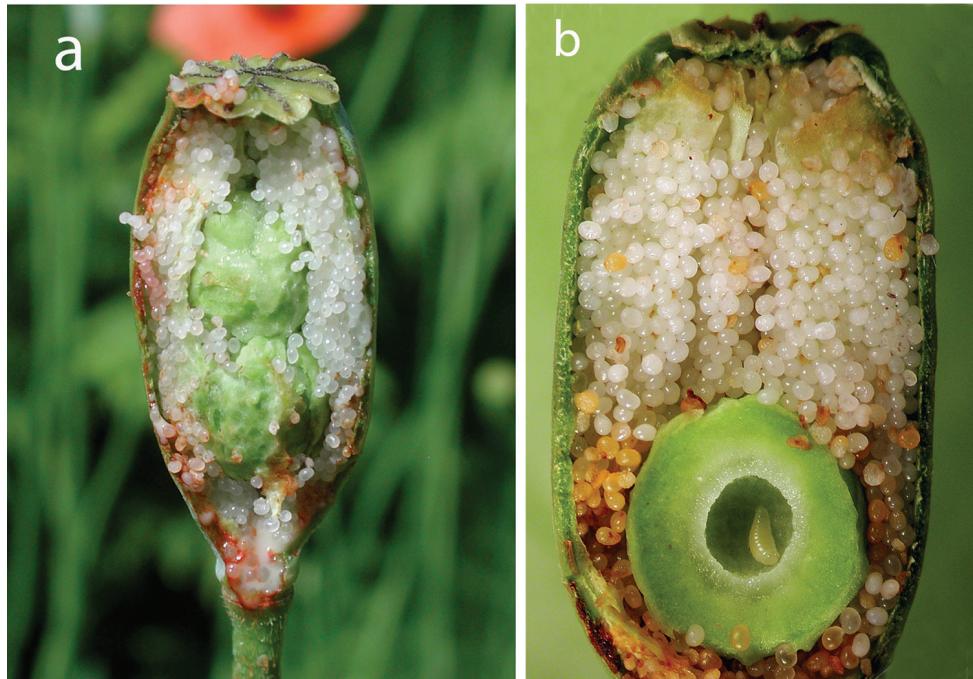
Figure 3. Young galls of *Barbotinia oraniensis* inside seed capsules of *Papaver rhoeas*. There may be 1–3, rarely up to 6–7 galls per seed capsule. The galls lie inside the seed capsule and are not connected to the capsule wall (**a**). A sectioned gall shows the thick layers of plant tissue surrounding the young larva (**b**).
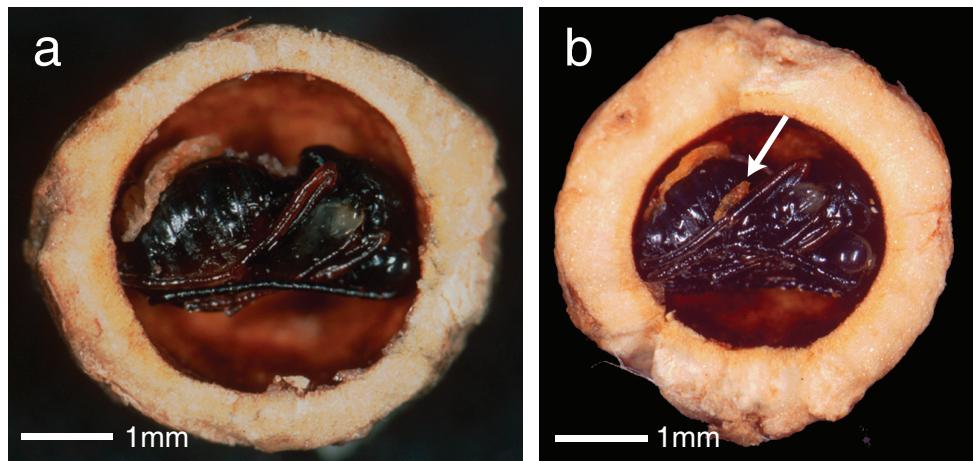

Figure 4. Galls inside the seed capsules of *Papaver rhoeas* opened in October may contain pupae of *Barbotinia oraniensis* (**a**) or *Parnips nigripes* (**b**). *Parnips* pupae are always found together with minute remnants of the terminal-instar larva of *Barbotinia* (arrow). Chambers occupied by healthy *Barbotinia* pupae do not contain remnants of other insects. Galls parasitized by *Parnips* are indistinguishable externally from normal *Barbotinia* galls but the wall is slightly thicker.
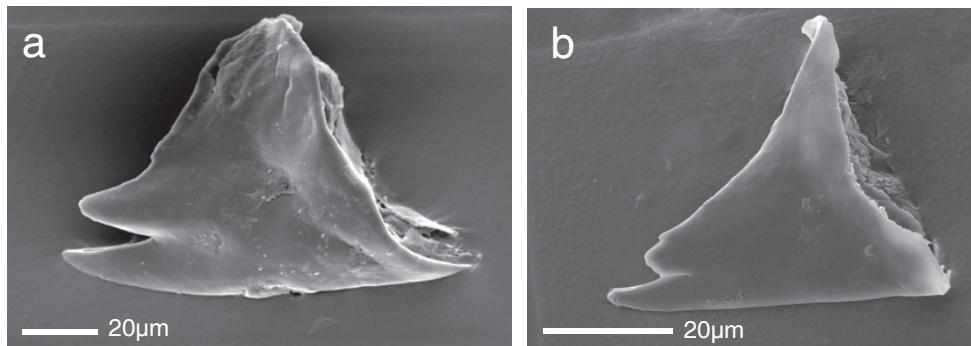
Figure 5. Mandibles of the terminal-instar larva of *Barbotinia oraniensis* (**a**) and *Parnips nigripes* (**b**). *Barbotinia* has a large mandible with two to three strong, blunt teeth. The mandible of *Parnips* is considerably smaller and has a single, elongate incisor with a weak secondary tooth along its upper margin.
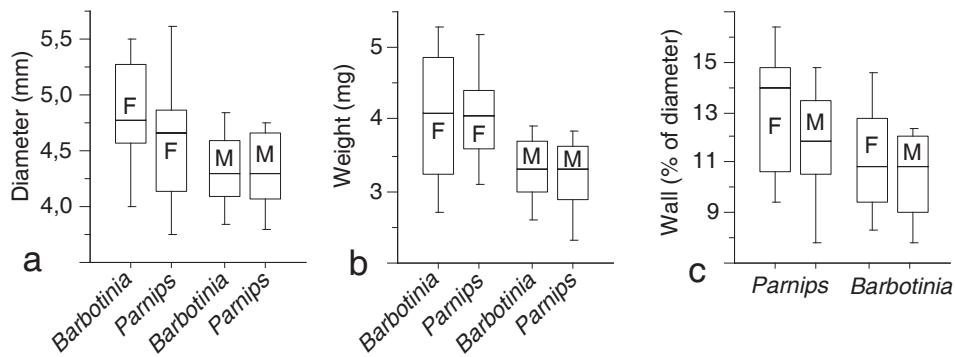

Figure 6. a Measurements of galls and pupae of *Barbotinia oraniensis* and *Parnips nigripes* (F = female, M = male). Galls containing females are larger than galls containing males (ANOVA: F = 8.075, df = 1, *p* = 0.006) but galls attacked by *Parnips* do not differ in diameter from normal *Barbotinia* galls (*p* = 0.51) **b** Female pupae are heavier than male pupae (F = 18.35, df = 1, *p* < 0.0001) but *Barbotinia* pupae do not differ in weight from *Parnips* pupae (*p* = 0.90) **c** *Barbotinia* galls attacked by *Parnips* have relatively thicker walls than normal galls (F = 6.98, df = 1, *p* = 0.01) both in females and males. *Barbotinia* females *n* = 20, males *n* = 18, *Parnips* females *n* = 27, males *n* = 15.

If the *Parnips* larva finishes its development by feeding on the gall tissue, as occurs in some parasitoids of gall insects, there should be less gall tissue left in galls containing *Parnips* pupae than in normal *Barbotinia* galls, assuming that *Parnips* is a less efficient gall tissue feeder than *Barbotinia*. We found the opposite. Galls containing *Parnips* larvae contained significantly more gall tissue (as indicated by the thickness of the gall wall) than *Barbotinia* galls (Fig. 6c, see also Fig. 4).

In our rearings, *Parnips* and *Barbotinia* specimens emerged simultaneously. We found that adults of both species survive for two to three weeks in room temperature with free access to water and sucrose solution. This is not sufficient time for the host larva to develop beyond the first one or two instars according to our observations (see below).

When offered young *Papaver rhoeas* plants, we observed several *Barbotinia* females ovipositing into small (2.0–2.5 mm long, *n* = 8) flower buds, which upon dissection were found to contain eggs or, after two to three weeks, minute larvae inside small galls. In contrast, *Parnips* females walked around on the plants but showed no interest in ovipositing. Unfortunately, we were not able to obtain sufficient material of both species in a single year to present *Parnips* females with plants containing eggs or young larvae of *Barbotinia*.

Parnips sp. B. The galls of *Iraella hispanica* are multichambered and occur in aborted flowers (Fig. 7a, b). Five young galls collected in 2003 at Gandesa were opened. They contained larvae of two different species, which we identified as *Iraella hispanica* and *Parnips* sp. B. Three galls contained two, four and five larvae, respectively, of *Iraella* and no larvae of *Parnips*. One of the remaining galls contained seven chambers with larvae of *Iraella*, two of which also contained a larva of *Parnips*. The final gall contained two chambers, both with a larva of *Iraella* and a larva of *Parnips*. When a larval chamber contained two larvae, the larva of *Iraella* appeared to be a mature or almost mature terminal-instar larva. In a couple of cases, the *Iraella* larva clearly seemed moribund. The larva of *Parnips* was attached to the surface of the *Iraella* larva in a position typical of ectoparasitic larvae (Fig. 7c). The *Parnips* larva was considerably smaller and more elongate than mature terminal-instar larvae of either *Iraella* or *Parnips* (Figs 7d, 8a). One *Iraella* gall collected in 2002 at Marça was found to contain a mature terminal-instar *Parnips* larva (Fig. 8b) but no larva of *Iraella*. The mandibles of this *Parnips* larva were similar to the mandibles of terminal-instar *P. nigripes* larvae (Fig 5b). Rearing of the collected but unopened galls (> 20 galls in total) yielded four males and two females of *Iraella*, and 19 males and 15 females of *Parnips*. No other insects appeared from the galls.

Discussion

Life-history of *Parnips*

Our observations reveal a number of interesting details concerning the life history of *Parnips nigripes*. The species is clearly an obligate parasitoid of *Barbotinia oraniensis*. Unlike many ectoparasitic larvae, the *Parnips* larva completely consumes its host, leaving just the cuticle and the mandibles of the host larva behind (Fig. 4). The close match in gall size and pupal weight between *Barbotinia* and *Parnips* indicates that the *Parnips* larva does not kill its host until late in the development of the latter, when the *Barbotinia* larva is fully grown or almost so. It is particularly interesting that the match in size appears to be sex-specific (Fig. 6). This suggests that ovipositing *Parnips* females actively match the sex of its offspring to the sex of the host. Such matching is known to occur in some other parasitic wasps (Godfray 1994). Future studies will have to show to what extent these observations also hold true for *Parnips* sp. B in galls of *Iraella hispanica*.
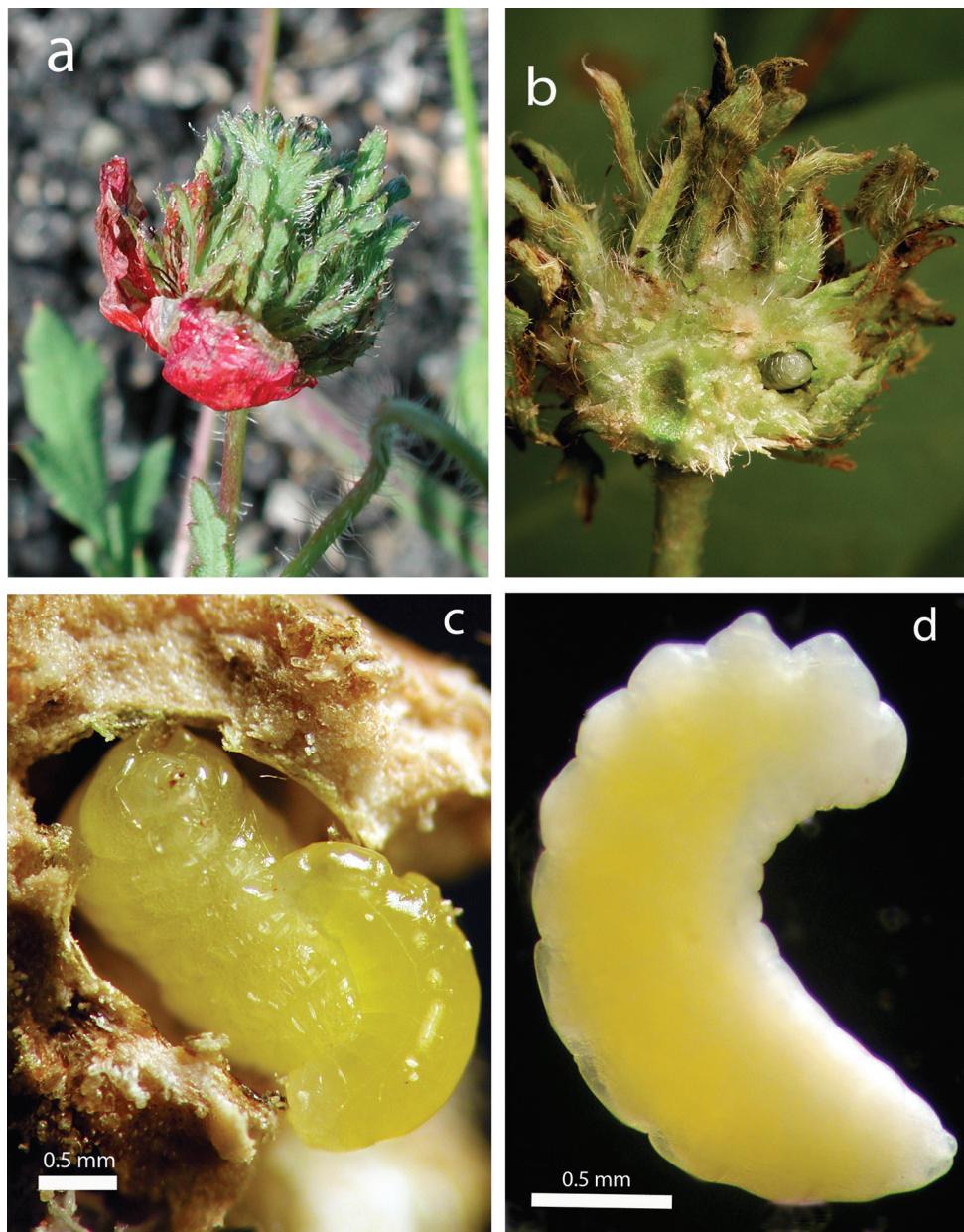
Figure 7. Young galls of *Iraella hispanica* in flowers of *Papaver rhoeas* and their inhabitants. **a** Gall **b** Transverse section of the gall showing gall chambers with larvae of *Iraella* **c** Mature terminal-instar larva of *Iraella* with an ectoparasitic intermediate-stage larva of *Parnips* sp. B. **d** Intermediate-stage larva of *Parnips* sp. B.

The mandibles of *Barbotinia* are clearly those of an herbivore, equipped with several strong and blunt teeth suitable for crushing plant cells (Fig. 5a), and the mandibles of *Iraella hispanica* are quite similar (see Nieves-Aldrey et al. 2005). In contrast, the

mandibles of both species of *Parnips* are characteristic of a carnivore with their single, sharp incisor (Fig. 5b). The shape and small size of the mandibles make the terminal-instar *Parnips* larva poorly equipped to complement its normal diet with gall tissue, if it can do it at all. It is true that there is some variation in the shape of the mandibles of gall inducers (Nieves-Aldrey et al. 2005), but the sturdy mandibles of both *Barbotinia* and *Iraella* indicate that the nutritional cells of poppy galls offer more chewing resistance than the *Parnips* mandibles can deal with.

The fact that *Barbotinia* galls containing *Parnips* pupae have distinctly thicker walls than those containing pupae of the gall inducer is interesting. A possible explanation is that *Barbotinia* larvae parasitized by *Parnips* lose their ability to accurately control the development of the gall towards the end of their life, resulting in some of the gall tissue that would normally have developed into nutritional cells remaining undifferentiated. In the normal development of cynipid galls, the thick wall of undifferentiated plant tissue surrounding the young gall successively develops into nutritional cells that are consumed by the cynipid larva (compare Figs 3 and 4). A possible alternative explanation is that the *Parnips* larva manipulates its host, such that the wall of the gall becomes thicker and provides better protection against hyperparasitoids. Such manipulation of host galls is known to occur in cecidomyid galls attacked by parasitic wasps in the genus *Platygaster* (Platygastridae).

Judging from the species that have been studied thus far, the life history of all cynipoid parasitoids is essentially the same (Ronquist 1999 and references cited therein). The egg is deposited inside a well-developed host embryo or a host larva, typically a young larva. Initially, the cynipoid larva stays inside the host, which develops normally. That is, it starts its life as a koinobiont endoparasitoid (Quicke 1997). Eventually, however, the cynipoid larva exits the moribund host and spends the last one or two instars feeding externally on the host remains. In all cases, this happens within a concealed space: a fly puparium, an aphid mummy, a lacewing cocoon, or the tunnel of a wood-boring host. Chrystal (1930) and Spradbery (1970) report that the *Ibalia* larva exits its host larva when the latter is fully grown, and then spends the third (penultimate) and fourth (ultimate) larval instars finishing it off, in the same way an ectoparasitoid would. This type of life history has been reported for a range of core figitids, as well as for the Ibaliidae (genus *Ibalia*) (see Fig. 1).

Although we have not been able to follow the entire life history of the two species of *Parnips*, our observations do suggest that they follow the typical pattern of cynipoid parasitoids. Clearly, the *Parnips* sp. B larva spends the latter part of its life as an ectoparasitoid. The fair amount of variation in size and shape that we observed in *Parnips* sp. B larvae (Fig. 8) could mean that there are two larval instars involved, as in *Ibalia*, but it is also possible that this is just the shape difference between newly hatched and mature terminal-instar larvae.

We have no observations of the early part of the *Parnips* life history, but circumstantial evidence suggests that *Parnips* is a koinobiont parasitoid during this phase. First, in our rearings where all galls and pupae were exposed to the same environmental conditions, adults of *Barbotinia oraniensis* and *Parnips nigripes* emerged simultane-
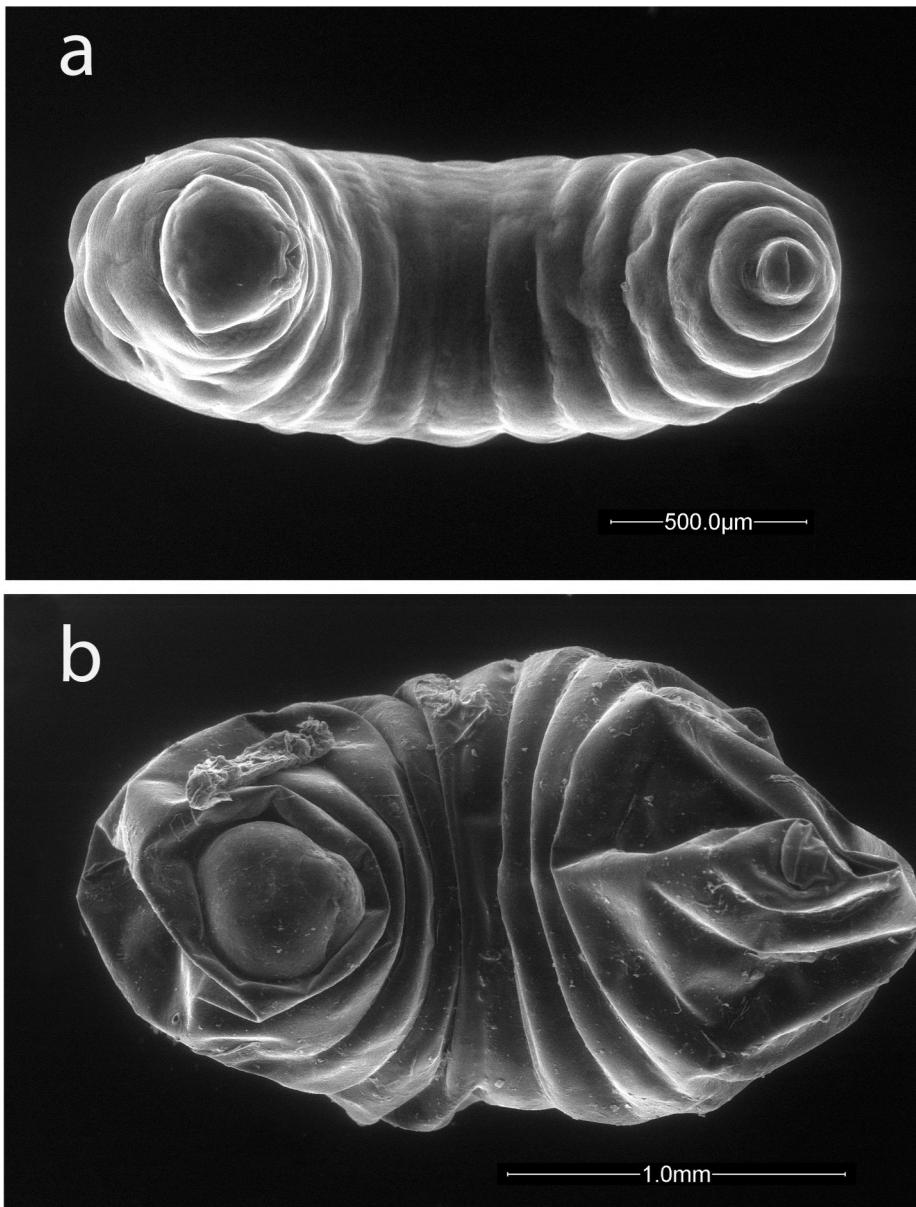
Figure 8. Scanning electron micrographs of an intermediate-stage (**a**) and a mature terminal-instar (**b**) larva of *Parnips* sp. B.

ously. This is consistent with the rearing data of Barbotin (1963) from Algeria, showing largely overlapping emergence periods. Second, our experiments show that the *Barbotinia* larva needs more than a few weeks before it is fully grown, probably 1–2 months by extrapolation, and they also suggest that the *Parnips* adults do not live more than a few weeks. Thus, *Parnips* females are unlikely to oviposit in or on mature terminal-

instar *Barbotinia* larvae. The *Barbotinia* larva clearly reaches the terminal instar before the *Parnips* larva kills it, as shown by the presence in all cases of the mandibles of the terminal-instar larva of *Barbotinia* in galls containing *Parnips* pupae. The similarity in size of normal and infected galls, and the match in pupal size between the host and its parasitoid, also suggest that the *Barbotinia* larva essentially needs to complete its development before it is killed by *Parnips*.

These observations indicate that *Parnips* is a koinobiont parasitoid during its early larval stages, like other cynipoid parasitoids. Most koinobiont parasitoids live inside their hosts (Quicke 1997), which would further support the conclusion that the life history of *Parnips* is the same as that reported so far from other cynipoid parasitoids. The conclusion is of course tentative, given that we still lack direct observations of the early phase of the life history, and further studies of the life history of *Parnips* are clearly needed.

Evolutionary origin of gall wasps

Perhaps the most significant aspect of the observations reported here is that they establish a link between the life histories of core figitids and ibaliids through the figitoid inquilines. This supports the hypothesis that gall wasps evolved from ancestors developing as parasitoids of other gall-inhabiting larvae. It also appears likely that these ancestors were koinobiont endoparasitoids in their early larval stages and ectoparasitoids in their late larval stages, like figitids and ibaliids. The hosts of these cynipid ancestors cannot have been gall wasps but must have been some other gall-inhabiting insects. The most likely candidates are perhaps gall-inducing or gall-inhabiting chalcidoids, as these appear to be the likely hosts of the extant figitoid inquilines that do not attack cynipid galls.

Hanson (1995) pointed out that most other groups of gall-inducing apocritans appear to have originated from ectoparasitoids of hosts that live inside plants, perhaps in many cases inside galls. He speculates that some of these ectoparasitoids started complementing the resources that they gained from the host larva by feeding on the gall tissue. Such facultative or partial herbivores might later have developed into obligate phytophagous inquilines, and eventually into true gall inducers. Essentially the same hypothesis was laid out independently by Ronquist (1995) for the origin of cynipids. A key difference between cynipids and other gall-inducing apocritans is that the cynipid ancestors appear to have evolved from forms that were endoparasitic in their early larval instars. If so, the origin of cynipid inquilines and gall inducers must have involved fairly dramatic changes in the early life stages of these ancestral forms.

It is currently unclear whether cynipid gall inducers evolved quickly from these ancestral parasitoids, or whether their origin was preceded by a protracted transitional phase of phytophagous inquilinism. Although early morphology-based studies supported the former scenario (Ronquist 1994, Liljeblad and Ronquist 1998, Ronquist and Liljeblad 2001), more recent molecular phylogenetic analyses raise the possibility that most or all cynipid inquilines represent survivors from an extended transitional

phase between parasitoids and gall inducers (Nylander et al. 2004, Ronquist et al. 2015; see Fig. 1).

What speaks against a slow transition is that it requires at least five independent origins of the ability to induce galls in cynipids (Ronquist et al. 2015, Ide et al. 2017; see Fig. 1). At first this may seem unlikely because of the spectacular complexity of cynipid galls, but most cynipid galls are actually fairly simple and the capacity to induce more complex structural modifications of the outer layer of the gall appears to have evolved only secondarily within a couple of clades of gall inducers (Ronquist and Liljeblad 2001, Ronquist et al. 2015). Since the recent discovery of a gall inducer deeply nested inside the inquiline tribe Synergini (Abe et al. 2011, Ide et al. 2017), we know that there must have been at least two independent origins of cynipid gall inducers. Why not more? We know that gall inducers have evolved at least 15 times in the Chalcidoidea alone (La Salle 2005). Another observation that might support multiple origins of gall inducers is the fact that many cynipid inquilines are able to significantly modify their host gall. An extreme case is *Synophrus politus*, another member of the inquiline tribe Synergini. It induces the formation of a large globular gall that bears no resemblance to the minute host gall, that of *Andricus burgundus* (Cynipidae: Cynipini; JLNA, unpublished data). Similar cases are known from *Periclistus* inquilines in the tribe Diastrophini, some of which can spectacularly modify their host galls (Shorthouse 1980). Perhaps these cases should be interpreted as evidence that the capacity to induce galls have evolved repeatedly in phytophagous inquilines, and not as support for the idea that cynipid inquilines are gall inducers that have lost the ability to initiate gall formation.

A difficulty with the idea that most extant cynipid inquilines trace their ancestry back to inquilinous forms that predated cynipid gall inducers is that most extant inquilines attack cynipid galls. A notable exception includes *Rhoophilus loewi*, apparently the sister group of the remainder of the Synergini, which is an inquiline in galls of the cecidosid moth genus *Scyrotis* on *Rhus* (Anacardiaceae) (van Noort et al. 2007). Other examples involve members of the Synergini that attack cecidomyiid galls on oaks (Askew 1999a, Wachi et al. 2011). It is possible that such associations with non-cynipids are more common than currently thought, and represent the ancestral state in the inquiline lineages.

Regardless of how fast the transition was, and how many times it occurred, the fact that cynipids originated from parasitoids of gall inducers has important implications concerning the selective forces driving the transition from parasitoids to phytophagous inquilines and gall inducers. The traditional hypotheses claim that galls evolved because they provide their inducers with enhanced nutrition, protection from hygrothermal stress, or protection from natural enemies (Price et al. 1987). If the ancestors of the gall inducers were parasitoids of other gall insects, and the first galls were similar to the galls that their ancestors attacked, none of these hypotheses would seem applicable. The ancestors were already protected from hygrothermal stress and developed on a diet (insect larvae) richer than gall tissue. The first gall inducers also likely suffered from the same enemies as their gall-inhabiting parasitic ancestors. Instead, it seems that the first step towards phytophagy involved the gain of the ability to complement the larval diet with gall tissue towards the end of larval development, perhaps because the

host sometimes died prematurely. Once this ability had evolved, one could imagine a ratchet-like process, whereby a gradual and irreversible loss of the capacity to keep the host larva alive throughout its development could be compensated for by an increased proficiency in surviving on gall tissue and keeping the gall developing despite the premature death of the gall inducer.

An intriguing possibility is that the association of *Parnips*, *Barbotinia*, and *Iraella* with Papaveraceae goes back to the ancestor of cynipids and figitids (Ronquist and Liljeblad 2001, Ronquist and Nieves-Aldrey 2001). Herbs in the Papaveracae occur as hosts in two tribes of cynipid gall inducers: Aylacini and Aulacideini (Fig. 1). Members of the former tribe, to which *Barbotinia* and *Iraella* belong, exclusively gall *Papaver*, while members of an early branch of the latter are associated with *Fumaria* and *Hypecoum*, belonging to another subfamily of Papaveraceae. Interestingly, *Chalcimerus borceai*, which we found to be the most common chalcidoid parasitoid of *Barbotinia oraniensis*, belongs to an early offshoot among the cynipid-parasitic torymids (Janšta et al. 2017). However, the most recent reconstructions of phylogenetic relationships suggest that the association with Papaveraceae in *Parnips* and its cynipid hosts is likely due to secondary convergence (Ronquist et al. 2015).

The more we learn about gall-inhabiting parasitic wasps, the more dynamic the evolutionary picture becomes. It seems that many of the gall-associated lineages include the full range of life histories: parasitoids, inquilines and gall inducers. Many galls also host members of several of the gall-associated hymenopteran lineages, and one can easily get the impression of an evolutionary relay race, in which the ability of inducing galls is passed on from one lineage to another in these communities. This idea fits nicely with the much-debated idea that gall induction involves a symbiont that could be transmitted horizontally across unrelated lineages (e.g. Cornell 1983). However, there is still no evidence of the existence of such symbionts, and it is possible that we instead should be looking for preadaptations to gall induction shared by these gall-associated apocritan lineages.

Conclusions

Although we have not been able to follow the entire life history, our observations clearly show that *Parnips nigripes* is a parasitoid of *Barbotinia oraniensis* and that *Parnips* sp. B is a parasitoid of *Iraella hispanica*. *Parnips* sp. B is ectoparasitic in the latter part of its larval development. Circumstantial evidence suggests that both species share the life history reported for other cynipoid parasitoids: koinobiont endoparasitoids in early larval instars and ectoparasitoids towards the end of their development. These findings imply that cynipid gall inducers evolved from parasitoids of gall insects, possibly through a prolonged intermediate phase of phytophagous inquilinism.

Clearly, we need progress on two fronts in order to gain a better understanding of the evolutionary origin of cynipid gall inducers. First, we need better resolution close to the root of the figitid and cynipid phylogeny, something that will hopefully come

from future phylogenomic analyses. Second, we need more detailed studies of life histories, not only of the figitoid inquilines but also of many cynipids. For instance, the life history of the cynipid tribe Ceroptresini is poorly documented and there is at least one report suggesting that it is a parasitoid and not an inquiline (Blair 1949). Similarly, the life history of Paraulacini is still unclear. Nieves-Aldrey et al. (2009) concluded that it is not a gall inducer and suggested that it might be a parasitoid of the chalcidoid gall inducer *Aditrochus*. This seems to be confirmed by recent genomic sequencing of *Aditrochus* larvae, which also picked up the Paraulacini genome (Nieves-Aldrey and Rasplus in prep.). Whether the life history of Paraulacini traces its origins back to the insect-parasitic ancestors of cynipids or represents a secondary reversal will remain unclear until the life history of Paraulacini is studied in detail. Clearly, there is more need than ever for careful study of the natural history of cynipids and their relatives.

Acknowledgments

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