



LARVAL STRATEGY OF TWO SPECIES OF SEED-FEEDING CHALCIDOIDEA PARALLELS THAT OF PARASITOID KOINOBIANTS

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Abstract: The association between ontogenetic processes in plants and phytophagous insects is not traditionally considered in studies of insect-plant interactions. Angiosperm seeds impose important constraints on seed predators; the structural complexity of seeds and the progressive accumulation of resources throughout their development limit the time windows when resources can be retrieved by the predator. Some holometabolous insects deposit their eggs inside immature seeds, with the immature stages of both insect and plant cohabiting in a space with limited but potential resources. We studied the larval development of *Megastigmus transvaalensis* (Hussey, 1956) (Chalcidoidea: Megastigmidae) and *Bephratelloides pomorum* (F., 1804) (Chalcidoidea: Eurytomidae) and the seed development of their respective hosts, *Schinus terebinthifolia* (Raddi, 1820) (Anacardiaceae) and *Annona crassiflora* (Mart., 1841) (Annonaceae). Our results show that both *M. transvaalensis* and *B. pomorum* oviposit in immature fruits, whose protective tissues surrounding the seeds are softer. The first larval instar interferes little with the development of the seed, allowing both seed and plant embryo to continue growing. When the infested seed reaches the size of a mature, non-infested seed, the larva grows rapidly and consumes most of it. *M. transvaalensis* induces minor modifications in the endosperm cells, while *B. pomorum* does not induce any visual modifications. The strategy of allowing seed/plant embryo to continue growing shows similarities to the endoparasitic koinobiont strategy followed by some chalcid parasitoids, which keep their host alive while feeding upon it. Future studies should be expanded to other chalcid seed predators in order to understand the evolution of convergent patterns among seed-feeding insects and its evolution in relation to parasitoid strategies inside the group.

Key words: chalcid wasps; larval development; phytophagy; seed development; seed predation.

INTRODUCTION

Insects have evolved a myriad of ways to exploit resources in terrestrial environments. Among them,

exploitation of plant tissue has been crucial for their evolutionary success (Bernays & Chapman 1994, Labandeira 1998, Labandeira 2007). Fossil records have shown two historical events relevant to the

evolution of the phytophagous habits of terrestrial arthropods (Labandeira 2007). The first one was the consumption of stems and sporangia occurring around 400 Mya. The second event, which took place around 320-330 Mya, was the consumption of other plant structures, such as roots, wood, leaves and seeds. A common pattern in these two historical events is that there were considerable time lapses between the emergence of the plant structure and its subsequent consumption by arthropods, suggesting that adaptation was necessary for the effective use of those resources. How the evolution of these strategies to exploit all and each of these structures occurred is a key question still being answered in the evolutionary history of insects (Bernays & Chapman 1994; Labandeira 1998).

The association between ontogenetic processes in plant structures and the use of resources by phytophagous insects is not traditionally considered in studies of insect-plant interactions. Morphological, chemical and physiological properties of the whole plant and its parts vary with ontogenetic stages, exerting selective pressure on insect traits related to how resources are used, how fast they are consumed and where and when the adult female deposits its eggs, all according to the developmental stages of the host (Kearsley & Witham 1989, Brennan *et al.* 2001, Lawrence *et al.* 2003). Indeed, the selective pressure exerted by the ontogenetic stages of plant structures can be considerable, to the point that it can affect the dynamics of entire insect communities (Straw 1989, Walz & Witham 1997).

Among plant structures, the seeds of Angiosperms have considerable ontogenetic and structural complexities that make them potentially difficult to exploit. The development of seed in Angiosperms involves complex inter-dependent processes of tissues and organisms with different origins and genomes; *i.e.*, the embryo (2n) and the endosperm (3n) deriving from two independent fertilization events, the nucellus and envelop tissues (2n) deriving from the mother plant, and the seminal chamber deriving from the female gametophyte (embryo sac) (Cocucci & Mariath 2004, Lersten 2004). Throughout its development, the seed gains size and nutritional value, rendering these resources more valuable at later developmental stages. In addition, the enveloping tissues harden, and many seeds accumulate deterrent or toxic

substances at later developmental stages (Cocucci & Mariath 2004), causing difficulty of access to these precious resources by outsiders.

Holometabolous insects have evolved strategies to circumvent most of the constraints imposed by this seed ontogenetic complexity. The most straightforward, and perhaps the most effective one, consists of the adult female laying her eggs in seeds that are close to maturity or completely mature, with the larva feeding on them (Serrano *et al.* 2001, Johnson & Romero 2004 Östergard *et al.* 2007). This strategy circumvents the constraints imposed by the complex embryological processes of the seed and ensures the availability of the total nutritious value of a mature seed to the offspring of the insect. In this case the larva feeds indiscriminately on the tissues and structures of one or several seeds. There is a weak selective pressure over traits favoring fine-tuned larval strategies linked to seed ontogenetic processes. Since many seeds accumulate deterrent or toxic substances (Fenner & Thompson 2005), selective pressures would be more related to coping with these chemicals for this particular strategy.

Conversely, the females of some hymenopterans, coleopterans and dipterans have evolved the strategy of ovipositing on immature seeds (Milliron 1949, Brody 1992, Takagi *et al.* 2010, Alves-Costa & Knogge 2005). In these insects, adaptive traits are expected to evolve responding to the need of the larva to exploit the potential resources of the immature seed without interfering with its ontogenetic processes. The development of the insect would be expected to reflect the selective pressures imposed by the ontogeny of the seed, with the insect adjusting its own development to that of the seed. In the present study we comparatively assessed the larval and seed development of two species of seed predator chalcid wasps (Hymenoptera, Chalcidoidea), each one attacking a specific Angiosperm species. In the superfamily Chalcidoidea parasitoidism of other insects and arthropods prevails in most species, with two discernible main strategies: koinobiont and idiobiont. Phytophagy is considered to be a derived trait, emerging independently at least 10 times in seven families (Heraty *et al.* 2013). Seed predation, in particular, has been reported in six families: Agaonidae, Eulophidae, Eurytomidae, Megastigmidae (formerly considered part of Torymidae), Pteromalidae, and Tanaostigmatidae. All except Agaonidae (*i.e.*, pollinating fig wasps

that feed on fertilized ovules), have an antagonistic relationship with their plant hosts. We intend to unveil the processes involved in the seed predation of two chalcid species from two of these families (Megastigmidae and Eurytomidae). Specifically, we assessed how larval strategies relate to the complex seed developmental processes and whether plant tissues inside the seeds were manipulated by the insects, given the ability of some species of phytophagous chalcids to manipulate plant tissue (e.g. gall formation; LaSalle 2005). Studying the processes behind the emergence of adaptations in seed predation will shed light on the possible selection pressures shaping the emergence of this habit in the group. Even more, assuming that symbiotic mutualistic interactions derive from symbiotic parasitic interactions, studying the constraints imposed by the ontogenetic processes of the seed could help solve the question of why, even though seed predation has emerged in six chalcid families, it only developed into mutualism in one of those (Agaonidae). Previous research demonstrated a synchronized development between the larva of agaonids and the affected seed, suggesting important developmental restrictions imposed by the ontogenetic processes inside the seeds of figs (Jansen-González *et al.* 2012). Studying in detail the larval biology of chalcid seed predators will contribute as well to elaborate better evolutionary hypotheses on the emergence of seed predation within this megadiverse group of predominantly parasitoid insects.

MATERIAL AND METHODS

We studied the wasp *Megastigmus transvaalensis* (Hussey, 1956) (Hymenoptera: Megastigmidae sensu Janšta *et al.* 2017) associated with *Schinus terebinthifolia* Raddi, 1820 (Anacardiaceae) and the wasp *Bephratelloides pomorum* (Fabricius, 1804) (Hymenoptera: Eurytomidae) associated with *Annona crassiflora* von Martius 1841 (Annonaceae).

Schinus terebinthifolia is native to Brazil, Argentina and Paraguay and, its individuals bloom all year long, with a peak between March and April when most of the individuals produce flowers; fruit development takes around 30 days (Lenzi & Orth 2004). The species is used worldwide as an ornamental and has become an important invasive species in many places (Grissell & Hobbs

1999, Lowe *et al.* 2000). The flowers are arranged in cymose inflorescences and the fruit is a drupe (one single seed per fruit).

Megastigmus transvaalensis is an alien, naturalized species (Scheffer & Grissell 2003) that consumes the seeds of *Schinus terebinthifolia* (Anacardiaceae) in Brazil (Perioto 1997). In Africa, *M. transvaalensis* is associated with species of *Rhus* (Anacardiaceae). Adult females of *M. transvaalensis* oviposit in immature seeds of *S. terebinthifolia*, the larva hatches inside the seed and consumes it; the pupa stays inside the seed and one single wasp emerges per seed. The wasp leaves an emergence hole on the fruit still attached to the plant (Perioto 1997).

Annona crassiflora Mart. is endemic to Cerrado physiognomy (Brazilian savanna). Its flowers are solitary or arranged in pairs, the fruit is syncarpic, with dozens of ovules per ovary, with several seeds per fruit. *Annona crassiflora* blooms from the end of September to December; fruit development takes around 3–4 months (De Melo 2009). Adult females of *Bephratelloides pomorum* oviposit in developing fruits of *Annona muricata*, *A. cherimola*, *A. montana* and *A. crassiflora*, and represent an important pest affecting several commercial cultivars (Grissell & Schauff 1990). The larva hatches inside the seed and feeds on it, the pupa stays inside, and one wasp emerges per seed; the emergence of the wasp usually occurs while the fruit is still attached to the plant. Damage to the fruit is indirect, caused by the wasp exit holes, which expose the fruit pulp to bacteria and fungi (Braga-Filho *et al.* 2007).

We studied *M. transvaalensis*/*S. terebinthifolia* in trees growing naturally on the campus of São Paulo University, Ribeirão Preto, Brazil (21°10'S; 47°48'W) and, *B. pomorum*/*A. crassiflora* in an *ex situ* orchard of fruit trees of Cerrado at the Federal University of Goiás, Brazil (16°35'S; 49°17'W).

For the *S. terebinthifolia* / *M. transvaalensis* system, experiments were performed independently on four *S. terebinthifolia* trees between January and March 2012. To study larval development, we exposed fruits in similar developmental stages to females of *M. transvaalensis*. Preliminary observations showed that females of *M. transvaalensis* oviposit on fruits within approximately 10–15 days after pollination (data not shown). Fruit development was synchronized by controlled pollination. To this end, about 200 flower buds were isolated with

organdy tissue bags before anthesis. Flowers/inflorescences were monitored daily until reaching anthesis and then hand pollinated. Pollen was collected from different individuals and used the same day in order to avoid self-incompatibility and ensure pollen viability. After pollination, flowers/inflorescences were re-bagged in organdy to avoid access by other insects.

Fruits produced by controlled pollination were exposed to female wasps for oviposition. Females of *M. transvaalensis* were previously reared from naturally infested fruits, with hundreds of mature *S. terebinthifolius* fruits being collected and kept in organdy bags in the laboratory for wasp emergence. Emerging females and males were collected with an entomological hand aspirator and kept inside organdy bags with cotton soaked in a water and honey solution; mating was allowed in the bags. Around 100–120 fruits were exposed to the mated female wasps; five to ten females were introduced in each bagged branch containing about 30 fruits. To monitor larval and seed development, we collected 10–15 fruits at random each 2–3 days.

For the *A. crassiflora*/*B. pomorum* system, fruit development was synchronized by controlled pollination. Flower buds were isolated before anthesis and hand-pollinated following the same procedures as described above for *S. terebinthifolia*/*M. transvaalensis*. Due to a low synchronicity among the flowers of a same individual, high fruit abortion rate and the need to ensure enough fruits of a single developmental age for rearing, flowers and fruits from nine *A. crassiflora* were used altogether in the experiment between October 2011 and January 2012. Around 60 flower buds were isolated from all the *A. crassiflora* individuals combined. Previous observations (data not shown) indicated that females of *B. pomorum* oviposit in fruits 50–60 days after pollination. Due to the difficulty of rearing enough female wasps in the laboratory, fruits were exposed to *B. pomorum* females that occurred naturally in the orchard. Organdy bags were removed for 48 h to expose 40 fruits to the wasps. Exposed fruits were monitored throughout the day to accurately verify which were oviposited by *B. pomorum* females. This was possible to determine by the direct observation of a female ovipositing in a fruit or by the identification of evident oviposition scars left on the surface of the fruit. Oviposited fruits were tagged with flagging

tape and re-bagged to avoid further access by insects. To study larval and seed development, we collected 2–3 *A. crassiflora* fruits at random each 4–7 days. Since not all the seeds of a fruit were affected by the wasp, unaffected seeds were sourced from oviposited fruits for comparison purposes. Affected seeds were easily identified because of ovipositor scars left on the exocarp. Collection intervals were defined according to larval size after dissecting a subsample of seeds from the previous collecting episode.

For both plant/insect systems samples of fruits up to the pupal stage were fixed for 24 h in FAA 50 (formalin: acetic acid: 50 % alcohol, Johansen 1940) and then transferred to a solution of 70 % ethanol. Each fruit was examined under a 10x magnification stereomicroscope and dissected to reveal the larva inside the seeds. The dissected seeds were photographed with a digital camera mounted on a Leica MZ16 stereomicroscope for description of larva/seed tissue development. We measured maximum length and width on a lateral view for eggs and larvae under a stereomicroscope using IM50 Leica™ software.

For the histological study, we subsampled groups of 10–15 infested and uninfested seeds at different intervals from the initial day of wasp introduction. Each material group was processed according to standard dehydration and softening protocols, embedded in Leica Historesin® (Gerrits 1991) and then sectioned with a Leica RM 2245 microtome into 5–6 µm sections. Serial sections were stained with 0.05 % toluidine blue, pH 4.4 (O'Brian *et al.* 1964) and slide mounted. Illustrations were taken using a digital camera coupled to a Leica DM 4500 microscope. All histological slides and wasp samples are in possession of R.A.S. Pereira (FFCLRP/USP) as voucher material.

RESULTS

By the time *Megastigmus transvaalensis* females oviposit (10–15 days after pollination) *S. terebinthifolia* fruits already attain full size (Figure 1a). At this stage, seeds are still small and underdeveloped, leaving a space between the fruit endocarp and the developing seed testa.

The wasp egg ($x \pm SD$: length = 0.228 ± 0.037 mm; width = 0.109 ± 0.014 mm; N = 19) is pedunculate, with a peduncle 2–3 times the total length of the egg

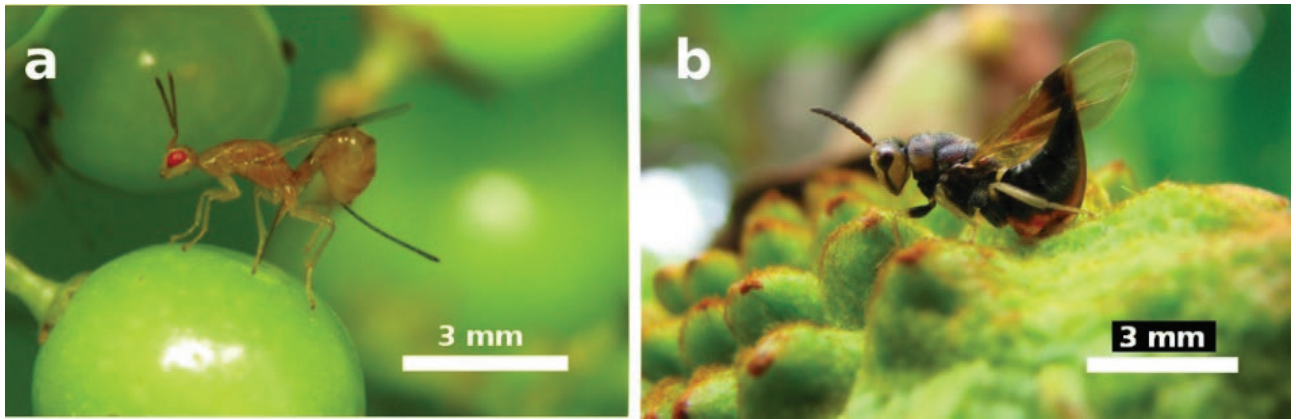


Figure 1. Females of, **a)** *Megastigmus transvaalensis* and **b)** *Bephratelloides pomorum* ovipositing on the fruits of their corresponding host plants.

(Figure 2a), and is deposited inside the endosperm, with the peduncle attached to the seed endotesta (Figures 3a–b). Wasp oviposition occurred in seeds containing an embryo at the globular, cordiform or early cotyledonary stages (Figure 3a). One to five eggs were observed per fruit.

Megastigmus transvaalensis has three larval stages, revealed by larval molts. The first-instar larva (length = 0.410 ± 0.133 mm; width = 0.216 ± 0.085 ; N = 20; 3–6 days after oviposition; Figure 2b) is located inside the endosperm and feeds on

it. The plant embryo is at the cotyledonary stage (Figure 3c). In the infested seed, the endosperm has hypertrophied cells (Figure 3c) when compared to uninfested seeds (Figure 3d).

The second-instar larva (length = 1.012 ± 0.375 mm; width = 0.411 ± 0.151 ; N = 25; 7–9 days after oviposition; Figure 2c) consumes the endosperm (Figures 4 a–b). At this instar the larva begins to consume the plant embryo. Both plant embryo and seminal chamber have doubled in size in relation to the seed where the first larval stage was present.

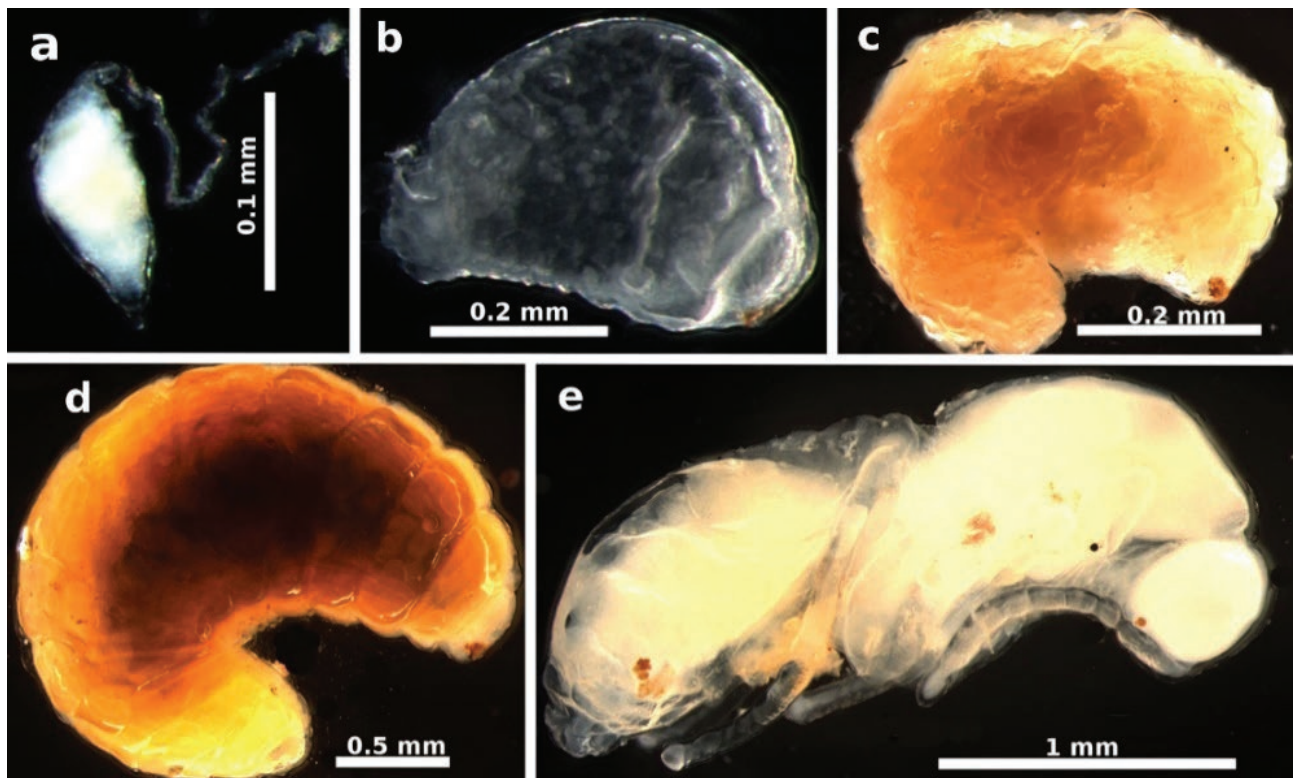


Figure 2. Immature stages of *Megastigmus transvaalensis*. **a)** Pedunculate egg; **b)** First larval stage; **c)** Second larval stage; **d)** Third larval stage; **e)** Early pupae.

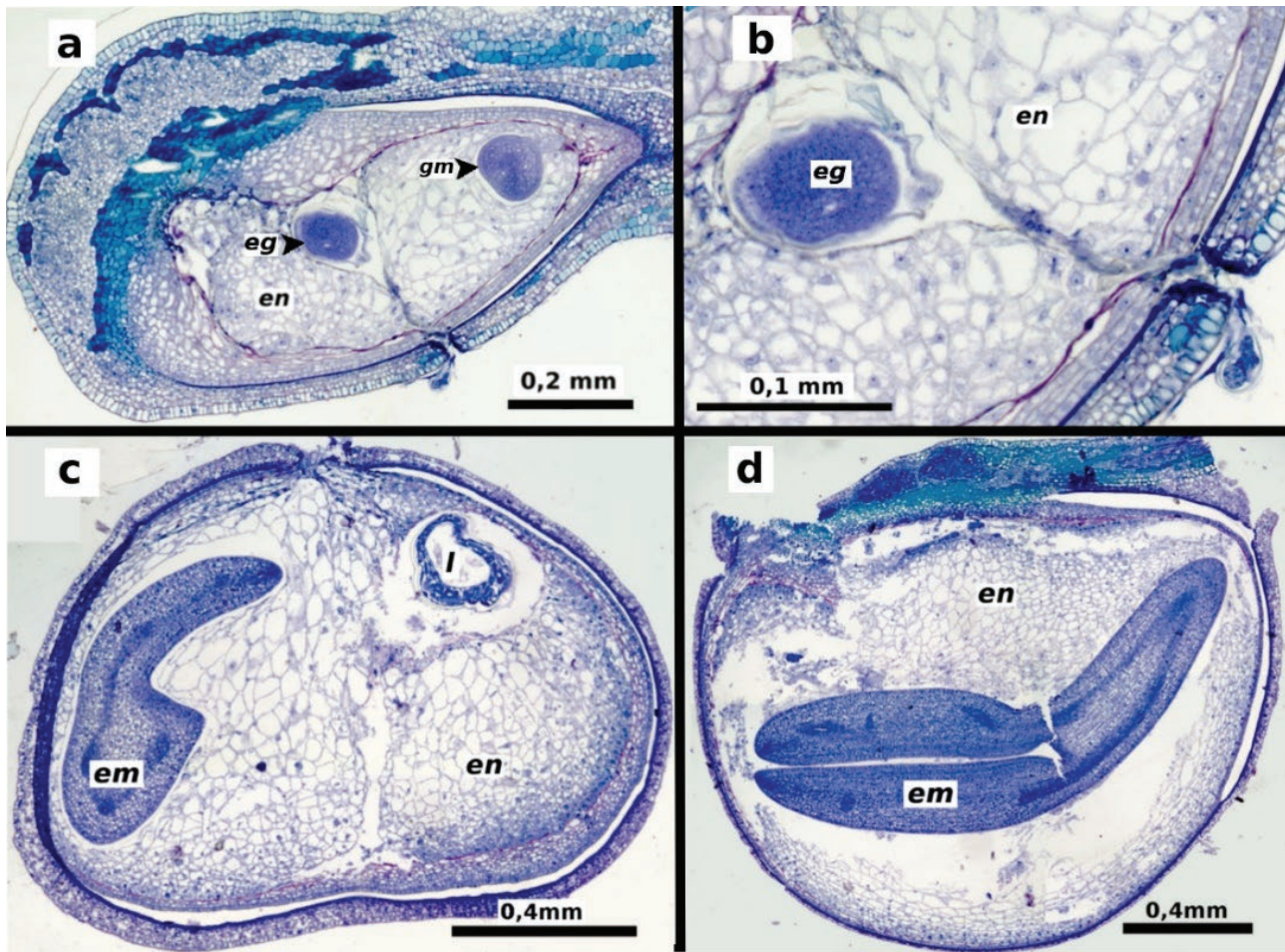


Figure 3. Longitudinal section of *Schinus terebinthifolia* seeds with and without immature stages of *Megastigmus transvaalensis*. **a)** Whole seed with wasp egg and plant embryo in the globular stage; **b)** Detail of a wasp egg inside the endosperm; **c)** Seed with a first larval stage; **d)** Non-infested seed. *eg* = wasp egg; *em* = plant embryo; *en* = endosperm; *gm* = plant globular embryo; *l* = larvae.

The third-instar larva (length = 3.427 ± 0.963 mm; width = 0.889 ± 0.218 ; N = 40; 10–15 days after oviposition; Figure 2d) feeds on the plant embryo and the rest of the endosperm (Figure 4c), consuming most of the seed. The seed chamber has grown to its total size. The larva reaches the pupal stage by 15–20 days after oviposition (Figure 2e).

Bephratelloides pomorum females oviposit in fruits 30–35 days after pollination (Figure 1b), the fruits being 8–10 cm in diameter, with seeds 0.8–1.0 cm in length. The egg (length = 0.372 ± 0.056 mm; width = 0.181 ± 0.025 ; N = 20) is pedunculate, with the peduncle reaching 3–4 times the total egg length (Figure 5a). The egg is deposited inside the endosperm (Figures 6 a–b), and its peduncle is attached to the seed tegmen. One to four eggs were observed per fruit.

Bephratelloides pomorum has four larval stages

revealed by the cephalic capsules attached to the larval body after each molt (Figures 5 d–e). The first-instar larva (length = 0.820 ± 0.170 mm; width = 0.136 ± 0.019 ; N = 12; 22–37 days after oviposition; Figures 5 b–c) is located inside the endosperm (Figures 6 c–d). At this time the seed houses a linear proembryo (Figures 6 e–f) and there are no perceivable differences between uninfested and infested seeds (compare Figures 6 c–d with Figures 6 e–f).

The second-instar larva (length = 2.361 ± 0.806 mm; width = 0.658 ± 0.242 mm; N = 12; 44–48 days after oviposition; Figure 5d) consumes the endosperm (Figures 7 a–b) or the plant embryo cotyledons (Figure 7c). At this time, the seed has considerably grown, and the endosperm has occupied most of the seminal chamber. By this stage the larva does not seem to interfere with seed development, as no apparent differences are

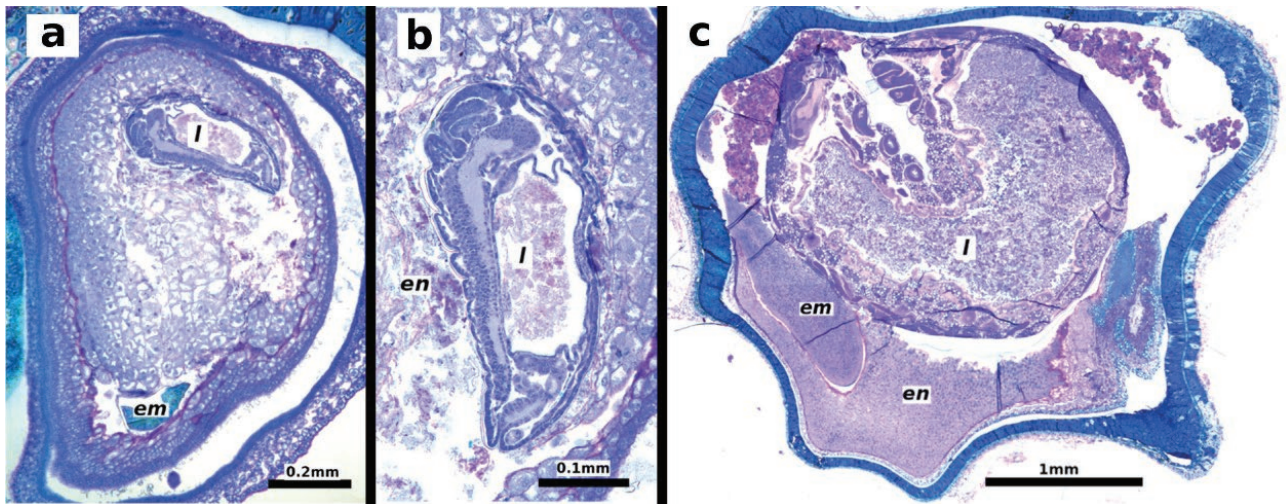


Figure 4. Longitudinal section of *Schinus terebinthifolia* seeds with a *Megastigmus transvaalensis* larva. **a)** Seed with a second larval stage; **b)** Detail showing a second larval stage inside the endosperm; **c)** Seed with a third larval stage. *em* = plant embryo; *en* = endosperm; *l* = larvae.

detected between uninfested and infested seed tissues.

The third-instar larva (length = 4.560 ± 1.119 mm; width = 1.335 ± 0.252 mm; N = 10; 51 days after oviposition; Figure 5e) continues consuming the endosperm (Figure 7 d–e). During consumption by the larvae there was no evidence of tissue induction or modification. By the end of this stage, larvae move close to the embryo radicle (Figure 7e).

The fourth-instar larva (length = 8.086 ± 1.515 mm; width = 2.366 ± 0.415 mm; N = 20; 55–61 days after oviposition; Figure 5f) consumes the whole plant embryo (Figure 7f) and pupates.

DISCUSSION

We presented detailed data on larva-plant interactions in two chalcid species that independently evolved the life strategy of feeding on developing plant seeds. Our results showed that *M. transvaalensis* and *B. pomorum*, despite belonging to distinct Chalcidoidea families, adopt a similar strategy by ovipositing in young fruits and adjusting their development to the resources that are still accumulating in the immature seed. The egg in both cases is deposited inside the endosperm from which the first larval instar feeds. The first larval instar has little impact on seed development, allowing both seed and plant embryo to continue growing. When the infested seed reaches the maximum size of a normal seed, the larva grows rapidly and consumes almost all the seed content. This strategy is also shared by

other seed-feeding chalcid species. Females of *Bruchophagus kolobovae* (Eurytomidae) oviposit in immature seeds of *Lotus corniculatus* (Fabaceae), even though consumption by the larva begins at more advanced stages (Batiste, 1967). *Megastigmus nigrovariegatus* uses a similar strategy in seeds of *Rosa rugosa* (Rosaceae) (Milliron 1949). Indeed, this strategy seems frequent in Megastimidae, especially in those species associated with Angiosperm seeds (Milliron 1949). In pollinating fig wasps (Agaonidae), although female wasps lay their eggs in pistillate flowers prior to ovary fertilization, initial larval development interferes little with fig embryogenesis, allowing the endosperm to grow while being consumed by the larva (Jansen-González *et al.* 2012).

In addition to chemical defenses, the access to the resources found in immature seeds is also constrained by plant embryogenesis, as many chalcid larvae rely on seed endosperm, as demonstrated here for two independent insect-plant partners and also observed in pollinating fig wasps (Jansen-González *et al.* 2012). Therefore, the behavior and ovipositor morphology of adult females, as well as larval development, are under selective pressures driven by seed development, as plant embryogenesis involves tissue growth and differential resource accumulation across the sporophyte tissues and genetically distinct organisms inside the seed (Cocucci & Mariath 2004).

The processes involved in seed predation by chalcid wasps are only partially known (Milliron

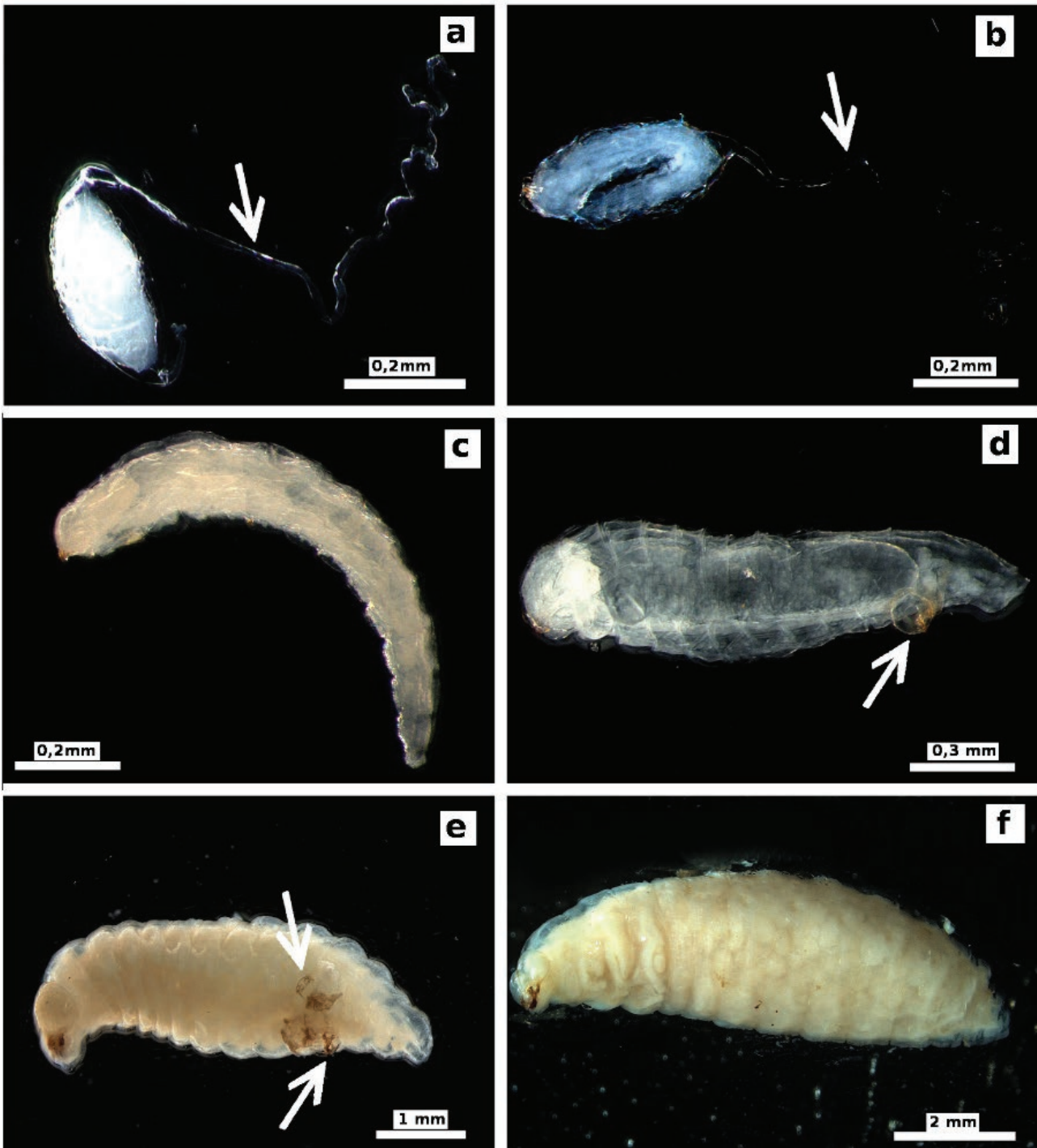


Figure 5. Immature stages of *Bephratelloides pomorum*. **a)** Pedunculate egg, arrow indicating the egg peduncle; **b)** First larval stage about to emerge, arrow indicating the remaining peduncle; **c)** First larval stage; **d)** Second larval stage, arrow indicating the molt head capsule; **e)** Third larval stage, arrows indicating the previous molt head capsules; **f)** Fourth larval stage.

1949, Batiste 196, but see Jansen-González *et al.* 2012, 2014). Studies of seed predation by insects, in general, have focused on the quantitative aspect of the interaction (*e.g.*, number of seeds predated) and its negative impact on plant reproductive success (Green & Palm bald 1975, Greig 1993, Herre & West 1997, Fenner *et al.* 2002, Fenner & Thompson

2005). Many insect lineages have evolved the strategy of using seeds as food resources for larval development, likely due to the higher nutritional value of seed reserves, especially those contained in the endosperm. The access to seed resources has a particular risk of being ingested within the seed by animal dispersers/granivores. A strategy

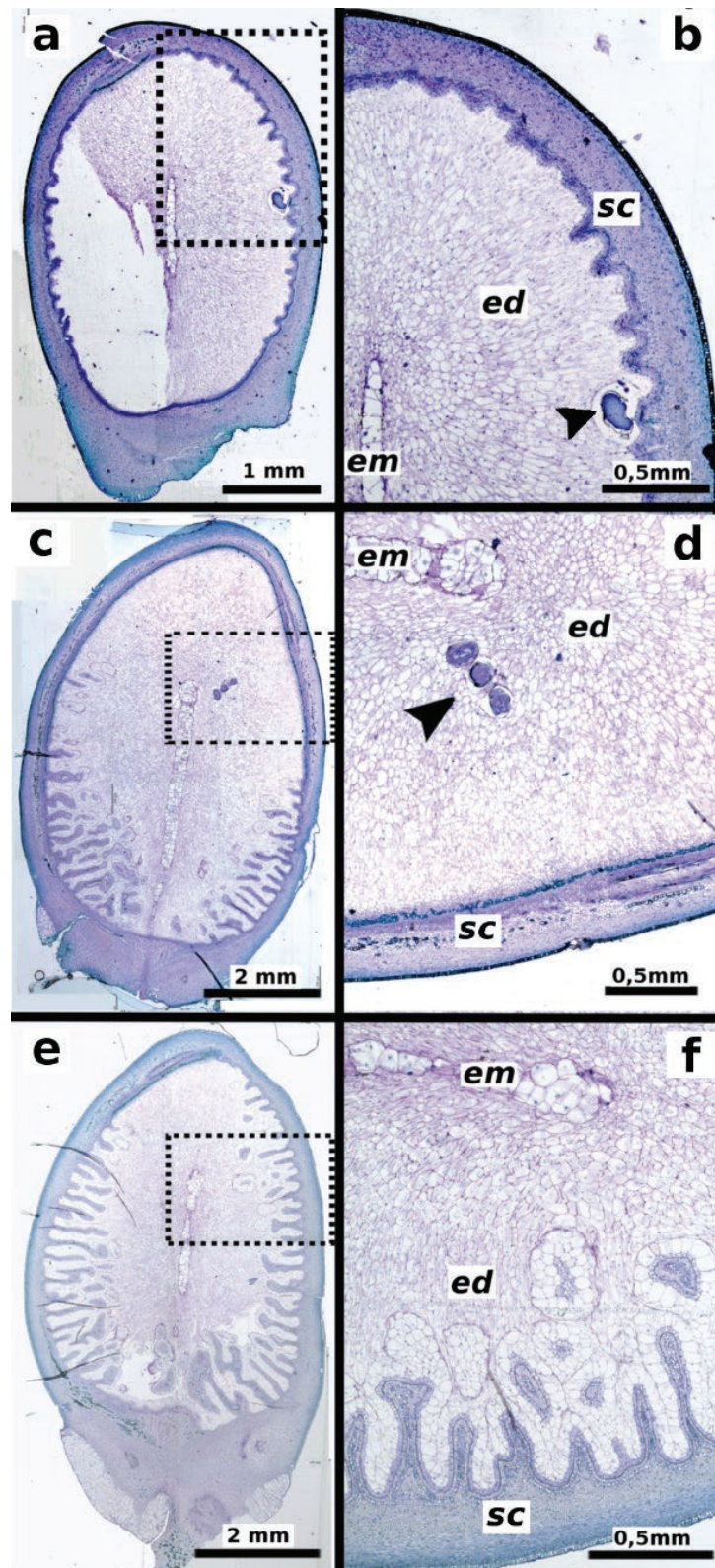


Figure 6. Longitudinal section of *Annona crassiflora* seeds containing or not immature stages of *Bephratelloides pomorum*. **a)** Seed with a wasp egg; **b)** Detail of the framed area in figure 6a showing egg location (arrow); **c)** Seed with first larval stage; **d)** Detail of the framed area in figure 6c showing location of the larva (arrow); **e)** Non-infested seed at same stage as in figure 6c; **f)** Detail of the framed area in figure 6e. *ed* = endosperm; *em* = plant embryo; *sc* = seed coat.

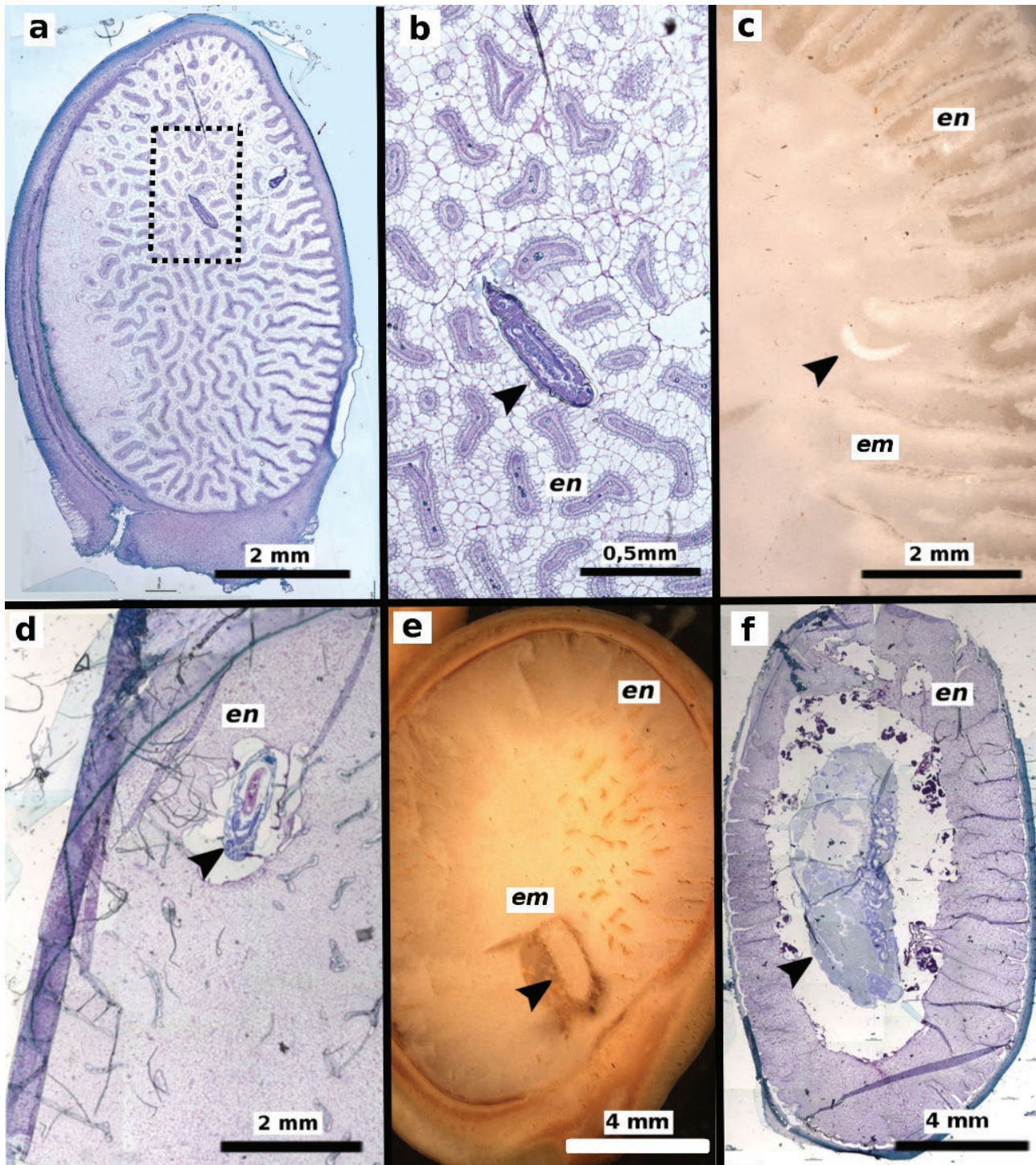


Figure 7. Longitudinal sections and dissections of *Annona crassiflora* seeds with *Bephratelloides pomorum* larval stages. **a)** Seed with second larval stage; **b)** Detail of framed area in figure 7a showing location of second larval stage (arrow); **c)** Dissected seed showing third larval stage (arrow) inside plant embryo cotyledons; **d)** Seed with third larval stage (arrow) inside the endosperm; **e)** Dissected seed showing later third larval stage (arrow) attacking the seed near the plant embryo radicle; **f)** Seed infested seed with a fourth larval stage (arrow); the larva has consumed most of the seed. *en* = endosperm; *em* = plant embryo.

to escape the predation risk adopted by chalcid species is to complete larval development before seed dispersal, which often leaves emergence holes in unripe fruits that are still attached to the plant (Milliron 1949; Batiste 1967). Additionally,

laying eggs in immature seeds might decrease the exposure of adult females to predators during oviposition, as the time spent boring through thin, soft fruit walls and seed coats is probably shorter than in mature ones (Ghara *et al.* 2011). Indeed,

predators of insects seem to exert a significant pressure on insect populations since it has been reported that the abundance of phytophagous insects affecting ripe fruits is inversely related to the abundance of avian dispersers, which are also potential insectivores (Herrera 1984).

Although the gross larva-host relationship was similar for *M. transvaalensis* and *B. pomorum*, we observed that *M. transvaalensis* modifies the endosperm cells, while *B. pomorum* apparently does not induce any tissue changes. This may indicate that what is often considered seed predation in Chalcidoidea might actually correspond to a spectrum of strategies used to feed on the reproductive structures of their host plants. Indeed, the life strategies of phytophagous chalcid species that feed on ovaries or seeds of their host plants might be more diverse than previously thought, blurring the division line between ovary parasites and seed predators. For example, some species have specialized in inducing galls out of structures of the ovary, such as the nucellus in the non-pollinating fig wasps *Idarnes* in *Ficus* (Jansen-González *et al.* 2014) or out of a combination of nucellus and ovary wall, such as we have found in a tetrastichine Eulophidae hosted by *Philodendron bipinnatifidum* (Jansen-González 2013). In the middle of the spectrum we would find strategies similar to those observed in *M. transvaalensis*, which induces inconspicuous galls on the endosperm, with imperceptible morphological modifications in seed and fruit. At the end of the spectrum we would have seed borers, such as *B. pomorum*, that produce no significant changes in seeds or associated reproductive structures.

Assuming that the phytophagous habit of Chalcidoidea derives from a parasitoid-entomophagous one (Heraty *et al.* 2013), considering seed feeding we can identify two main complex traits of chalcids that have allowed them to conquer this niche. First, the complex structure of chalcid ovipositors (Quicke *et al.* 1994, Le Ralec *et al.* 1996, Elias *et al.* 2018), which allows precise maneuvers to locate and access hidden hosts (*e.g.*, wood-boring larvae and leaf/stem galls), permitting the multiple shifts to the seed-feeding strategy in the group. Second, the well known ability of parasitoids to circumvent the host defenses and manipulate its physiology for its own benefit (Vinson & Iwantsch 1980, Pennachio

& Strand 2006) could be a determinant factor for chalcids to evolve the strategy of feeding from developing seeds, where the ability to circumvent developmental and physiological constraints from the host is important as well, as shown by both species in this study.

The strategy of allowing seed/plant embryo to continue growing, observed in *M. transvaalensis*, *B. pomorum* and pollinating fig wasps (Jansen-González *et al.* 2012), has some similarities to the endoparasitic koinobiont life history of some chalcid parasitoids (Vinson & Iwantsch 1980, Pennachio & Strand 2006). As more biological information accumulates in hand with better resolved phylogenies of Chalcidoidea, hypotheses on the possible evolutionary relationships between the koinobiont parasitoid habit and seed predation can be tested. At least two possible scenarios can be hypothesized, either the aforementioned similarities indicate that the koinobiont parasitoid habit was a prerequisite for the emergence of the seed predation strategy revealed in *M. transvaalensis* and *B. pomorum*, or convergent strategies emerged in both koinobionts and these seed predators in order to solve similar problems (*i.e.* access to resources in underdeveloped hosts). Several limitations hamper the testing of these hypotheses in the groups to which *M. transvaalensis* and *B. pomorum* belong (Megastigmidae and Eurytomidae). Megastigmidae is a monophyletic, phytophagous group, recently proposed to be elevated to family level (previously included as a subfamily of Torymidae) (Janšta *et al.* 2017) and needs to be studied further to reveal its position inside Chalcidoidea. For Eurytomidae, phylogenies still lack most of the groups (Lotfalizadeh *et al.* 2007) and both idiobiont and koinobiont strategies are present, which requires a well resolved phylogeny in order to understand the evolutionary relationships between parasitoidism and phytophagy inside the group. Future studies should expand the scope to other seed predator chalcids, as well as other Hymenoptera (*e.g.* Ichneumonoidea; Macêdo *et al.* 1998) and insect orders (*e.g.* Diptera; Straw 1989) in order to investigate convergent patterns among seed-feeding insects and to understand why and how some mutualistic interactions between insects and plants involve a seed predator that is at the same time a pollinator.

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