Clinical Microbiology: Open Access

Giangaspero and Turno, Clin Microbiol 2015, 4:5
DOI: 10.4172/2327-5073.1000e128

Editorial | Open Access

Aethina tumida, an Exotic Parasite of Bees

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Received date: September 01, 2015; **Accepted date:** September 07, 2015; **Published date:** September 14, 2015

Abstract

The small hive beetle (*Aethina tumida* Murray), pest of honeybees, diffused from sub-saharan Africa in different countries, and has become a serious issue for beekeepers. Several methods have been developed to control this pest. Optimization of control measures, and possibly successful eradication measures against this invasive coleopteran will contribute to contain the general problem of the decline of *A. mellifera* worldwide.

Keywords: *Aethina tumida*; Control; Honeybee; Pest; Small hive beetle

Editorial

The small hive beetle (*Aethina tumida* Murray) (SHB), coleopteran of the family *Nitidulidae*, is a scavenger and parasite of colonies of honeybee (Figure 1) [1]. There are some reports of infestation of bumble bee species (*Bombus terrestris* and *B. impatiens*) [2]. *A. tumida* was previously known only from the sub-saharan regions of Africa. This exotic pest of bees showed the potential of a trans-boundary disease diffusing out from native areas, and has become a serious issue for beekeepers in different countries. Although it is considered to be a minor pest of bees in Africa, it is a major problem in areas where it has been introduced. In Africa, the native honeybees are able to control the infestation by eliminating the majority of beetle larvae. At the contrary, this behavior is not observed among european honeybees therefore infestation develops and constitutes threat for the colony. Bees seem to counter the action of the insect by imprisoning and blocking movements as first defense against beetle reproduction. However, this reaction is not sufficient to prevent predation. The bees even feed the beetle, responding to solicitations made by the parasite (one response every 12 solicitations). Highly likely to be transported internationally accidentally, due to the proved invasive character of the species outside its native range, with high reproductive potential and highly mobile locally, the risk of impact outcomes, predation, damages to apiaries and bees products, resulting in general negative impacts on agriculture and livelihoods, the small hive beetle infestation is on the list of diseases notifiable to the World Organisation for Animal Health (Office International des Épizooties: OIE) [1].

The adult beetles of *Aethina tumida* are dark brown to black and about one-half centimeter in length and 3 mm wide. The insects move quickly, prefer to escape from the light and seem insensible to cold [3,4]. The adult beetles are able to fly several kilometers (up to 13 km) [2], aiding the rapid spread of infestation. The adults are attracted to bee colonies to reproduce. Female beetles lay irregular masses of eggs in cracks or crevices in a hive. The eggs hatch in 2-3 days into elongated, white-colored larvae with small spines in rows along the back [5]. Larvae will grow to 10-11 mm in length and require about 10-16 days maturing. Larvae that are ready to pupate leave the hive and burrow into soil near the hive to complete their cycle. Sandy and humid soil is most favorable [6]. The pupation period may last approximately 3-4 weeks. The pupae are whitish brown [3]. Newly emerged adults, red, but quickly becoming blackish, seek out hives and females generally mate and begin egg laying about a week after emergence. The adults may live up to 6 months. Hive beetles may have 4-5 generations per year under suitable conditions [3,7]. The small hive beetle can be a destructive pest of honeybee colonies, causing damage to comb, stored honey and pollen. Adult beetles and larvae feed on honey, pollen, honeybee eggs and larvae, dead adult bees, and combs. The beetles can also be a pest of stored combs, and honey (in the comb) awaiting extraction. The primary damage to colonies and stored honey caused by the small hive beetle is through the feeding activity of the larvae. While feeding, beetle larvae tunnel through wax comb with stored honey or pollen, damaging or destroying cappings and comb. Larvae defecate in honey and the honey becomes discolored from the feces. Activity of the larvae causes fermentation and frothiness in the honey, developing a characteristic odor of decaying oranges and, thereby rendering the honey foul, unpalatable to bees and considered unfit for human consumption. Hives and stored equipment with heavy infestations of beetles have been described as a mess. Various damages are caused by these beetles. Damage and fermentation cause honey to run out of combs, creating a mess in hives or extracting rooms. Its presence can also be a marker in the diagnosis of colony collapse disorder for honeybees. Heavy infestations may cause bees to abscond and desert their hives [3,8].

In the African continent it has been reported in different countries, first in Zimbabwe in 1964 by Papadopoulo *et al.* [9], then in Uganda [10], Sudan [11], Kenya [12] and Nigeria [13]. The life cycle information is known primarily from studies in South Africa [14]. An alleged introduction in 2000 in Egypt was reported but not substantiated due to unreliable record [15]. From Africa, the pest has been further introduced to other continents. The United States first suffered from a wide diffusion of *A. tumida* out of native areas. Since its initial discovery in 1996 and its subsequent identification in 1998, from a commercial apiary in St. Lucie County, Florida, it was soon detected in indian river and 4 other counties, where it caused significant damage to honeybee colonies [16].

Figure 1: European Honeybee (*Apis mellifera* Linneaus) is natural host of the small hive beetle (*Aethina tumida*). The European honeybee is particularly susceptible to the infestation suffering from heavy losses. (Photo by Chiara Giangaspero).

Beetle specimens were found in beehives near Atlanta, Georgia [3]. The intra-or interstate movement of honeybee colonies was prohibited until they could be inspected, to prevent further spread [16]. However, the species spread rapidly and is now very widespread throughout various other States in the USA, including North and South Carolina, California, Michigan, Pennsylvania, Ohio, Illinois, New Jersey, Minnesota, Maryland, Missouri, New York, Connecticut, Virginia, Texas, and also in Hawaii, where it was first recorded in 2010. In Canada, the small hive beetle has been detected in Manitoba (2002 and 2006), Alberta (2006), Québec (2008 and 2009), and Ontario (2010). In the Prairie Provinces (Manitoba and Alberta), measures were taken to control the pest and small hive beetles failed to establish a population. In Ontario, the introduction is restricted to a quarantine area in the southern part of the State. It is still to be determined whether the small hive beetle has been able to establish a resident population in Québec [17-19]. Furthermore, it has been also confirmed in Jamaica and Mexico in 2005 and 2007, respectively [15]. In Austral Asia, it was first found in Richmond, New South Wales and Queensland, Australia, in October 2002 [20], and is now considered endemic in those states, with some beetles recorded in Victoria and Western Australia [15,21]. Furthermore, a first SHB case was documented in the Philippines in June 2014. Inspection of apiaries in Lupon, Davao Oriental and General Santos in southern Mindanao Island in November 2014 revealed severe infestation, and majority of the bee colonies collapsed [22]. In Europe, a first observation was made in Portugal in 2004. The beetle was intercepted at a border inspection post in a consignment of bees originated from Texas [23]. In this circumstance, the prompt seizure of contaminated bees and subsequent destruction prevented the introduction of the pest. In September 2014, the introduction and natural reproduction was first recorded in Southern Italy. The presence was localized in the regions of Calabria and Sicily [24]. To December 2014, since the first detection, the parasite was found in 59 loci in Calabria (56 in the province of Reggio di Calabria and 3 in the neighboring province of Vibo Valentia) and in one migratory apiary in the province of Siracusa, in Sicily. Larvae of SHB have been detected in only 5 hives, out of a total of 60 infested hives, and in one case, nymphaes have been collected in the soil. All the hives confirmed positive to SHB have been destroyed. To July 2015, 782 apiaries have been examined within and out of the different established protection and surveillance zones in Calabria and 318 in Sicily. There has been no *A. tumida* outbreak observed since December 2014. The current distribution of the parasite is schematically presented in Figure 2.

The small hive beetle can spread in various ways including with honeybee colonies, honeybee queens, bee products, equipment and supplies and, very significantly, ripe fruit [25]. Sea ports are therefore a significant potential point of entry. The movement of migratory beekeepers from Florida may have transported the beetle to other states [3]. Recent findings also indicate transport of the beetles in package bees, but also by pollen and wax. In Manitoba, Canada, the parasite was introduced through imported beeswax from USA [17]. In Australia, it is suspected that a combination of importing queens from other countries and beekeepers moving their hives has caused the spread [26]. The mode of arrival of SHB in Southern Italy is unknown. However, it is meaningful that the first occurrence has been reported near the international sea port of Gioia Tauro in sentinel hives placed by the Department of Agriculture of the Mediterranean University of Reggio Calabria for early detection of eventual introduction of exotic bee pests onto the national territory. Furthermore, risk of introduction in new apiaries seems to be apiary density, beetle population levels, and ongoing mass beetle reproduction. Apiaries in forested habitats showed higher infestation levels, which was thought to be due to the presence of wild/feral colonies [27].

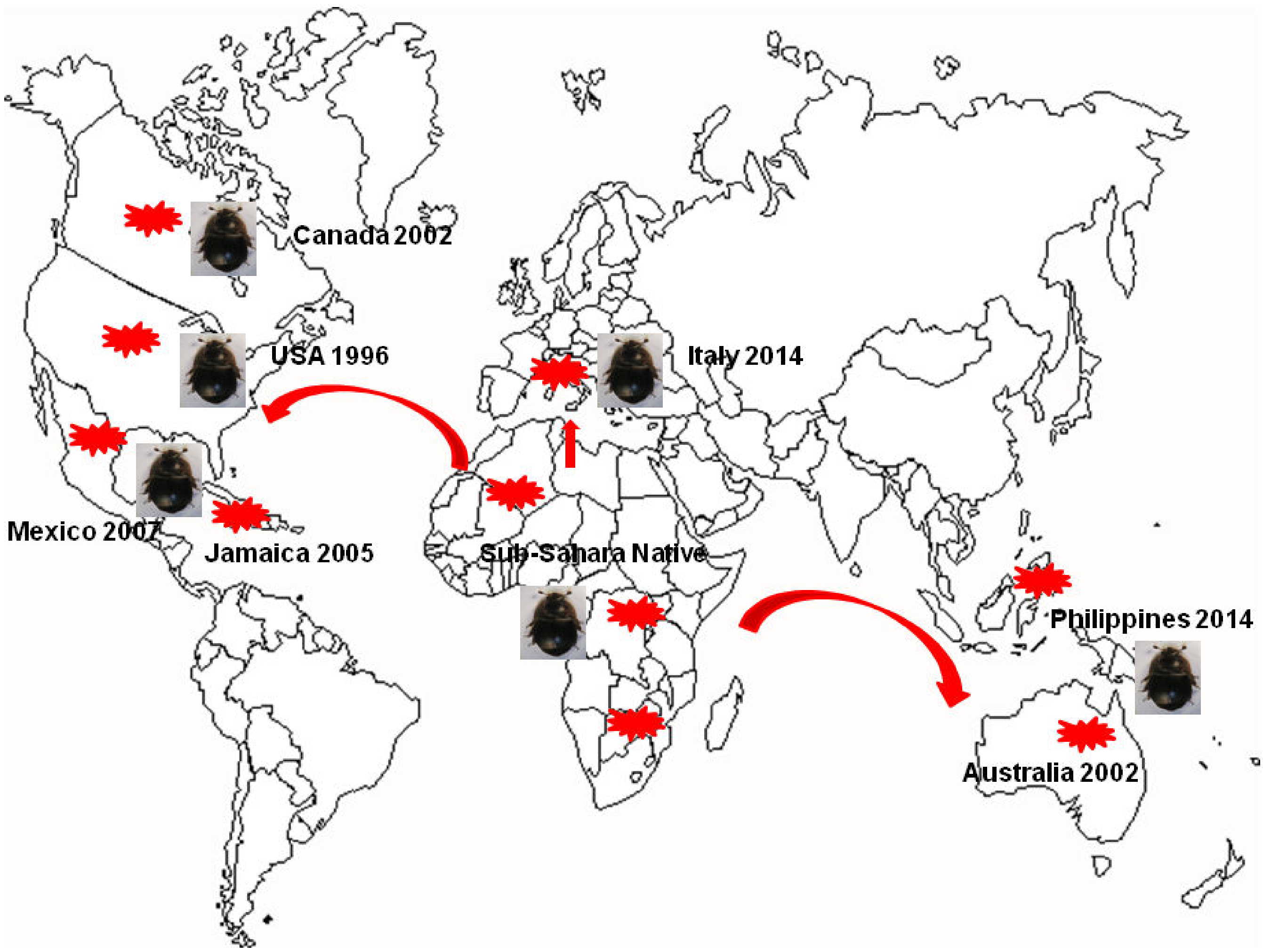

Figure 2: World distribution of the small hive beetle (*Aethina tumida).* The coleopteran, native from sub-saharan Africa spread in other continents. Year of first report is indicated.

Since this non-native species of beetle poses a major threat to the bee industry, this calls on the competent authorities to strengthen quarantine and other bio-security measures to prevent its spread. Policies on quarantine and bio-security measures should be streamlined in order to counteract the SHB threat. These should include movement control, prohibiting movement of bees from affected zones, controls at border inspection posts and field monitoring. SHB has been made notifiable within the european community in 2003 by Commission Decision 2003/881/EC [28]. In Canada and Australia beekeepers are to report immediately on learning of the occurrence of, or suspecting the existence of infestation by *A. tumida* [29,30]. However, despite in USA SHB is a serious widespread problem; *A. tumida* is monitored but not notifiable, as all other bee diseases [31]. Field monitoring to detect the presence of *A. tumida* can be supported by the installation of traps to capture adults in the hives [32]. However, traps are less efficient for the detection of adults than visual inspection of colonies, especially when the number of adults of *A. tumida* is very low. In Italy, during visits of five colonies resulted contaminated, adults have been collected in only two traps [24]. Therefore, it is important to inspect the entire hive to detect predators. Adults can be observed almost anywhere in a hive, although they are most often found on the rear portion of the bottom board of a hive. Each frame should be carefully and rapidly examined. Between the 20% to 50% of the colonies should be visited [24]. Subsequent identification of the collected insects by competent entomologists will avoid misdiagnosis with other resembling coleopteran as *Meligethes aeneus*, parasite of rapeseed, occasionally present in hives. The larvae of *A. tumida* may be confused with those of the wax worm (*Galleria mellonella*). A method to screen hive debris for the presence of SHB using real-time PCR in conjunction with an automated DNA extraction protocol is also available [33].

Several methods have been developed to control this pest. Chemical methods may be used in case of heavy infestation. Due to toxicity, risk of development of resistance, and potential residues in honey and in the environment, the use of insecticides to treat infestations should be carefully managed. Only authorized pharmacologically active substances, regularly registered as veterinary drugs should be used. Paradichlorobenzene (PDB) has been used for protecting empty stored combs. Coumaphos bee strips (Check-Mite+®, Bayer Corporation), organophosphate pesticide, have been approved for use in hives for the control of small hive beetles in USA and Canada (registered against varroasis in Europe as Perizin®). Strips with 10% of Coumaphos are placed in the dark, under corrugated cardboard squares at the bottom of the hive, were adult beetles are more likely to seek refuge [34,35]. Other miticide molecules registered for varroasis as tau-fluvalinate or amitraze should be effective also against SHB. Taking into account that larvae burrow into soil between 1 cm and 20 cm of deep to pupate near the hive, a 40% permethrin solution (Gard Star®, Y Tex Corporation) may be shed onto the soil for the destruction of larvae and nymphs to stop the reproduction cycle [36]. Good results may be obtained also with combined utilization of strips with 10% of Coumaphos in the hives. Boiling water may be also used on the soil around the hives. However, this method is not totally efficient since despite the majority (83%) of larvae remains at less than 30 cm of the hive, 17% at 90 cm, and no one is generally observable at 1.80 m from hive [37], SHB can crawl large distances (>100 m) to find suitable conditions for pupation [21] and, they may proceed even up to five hundred meters before burrowing and complete their cycle [8].

A good management by beekeepers is necessary to protect against *A. tumida*. Maintenance of strong colonies and good husbandry are key elements [38]. The beetle is most often found in weak or failing hives and rarely affects strong hives, reproducing faster when the bee population is insufficient. Therefore, the most effective control against small hive beetle is maintaining colony strength, coupled with minimizing empty frames of comb. Healthy colonies, with young and vigorous queens, will be able to withstand the SHB invasion and to control their numbers in the hive. As the maintaining of colonies healthy is vital, it is even more important to treat for varroasis and ensure honeybees are as healthy as possible. However, some beekeepers have reported the rapid collapse of even strong colonies. As part of good management, manipulations should be reduced to essential necessary. It seems that adults of *Aethina* invade preferably hives after opening for routine works, in relation with an attraction provoked by odors from the hive [34,39]. In SHB, reactivity to odors emanated from the hive increases with the number and age of worker bees. Females are more reactive to odor stimulations, with a maximal response to honey under elaboration in comb cells. Thus, routine tasks carried out by apiarists can aid the spread of beetles and their establishment in the hive [3]. Furthermore, maintenance and hygiene of hives should not be neglected. During spring visit, all old wax, propolis and any other debris that may constitute an egg laying zone for SHB should be eliminated and burned. Wooden structures should be flame heated by blowtorch, and all parts and frames washed with diluted bleach [40].

Taking into account that bees show more precision in their flying and landing capacity than *A. tumida*, assays have been undertaken to modify access to hives in order to prevent the introduction of the beetles. In a preliminary study, the hive entrance was closed and replaced by a plastic tube of 19 mm diameter and about 10 cm long, located at 8-10 cm above the landing board [41]. The number of beetles has been significantly reduced in the hive, however the new opening system caused decrease of bee brood, increase of debris volume (more difficult to evacuate) and the quantity of water present in the hive due to bad ventilation. Such side effects have been partially attenuated by using bottom grids instead of conventional non ventilated plain wood bottoms and larger entrance [42]. A grid with 2 mm mesh impedes the passage of SHB adults [43]. Similar methods have been applied by Hood and Miller [44].

There are also several in hive mechanical traps currently available that can alert beekeepers to the presence of SHB and help to control its population, while also reducing the need for pesticides. The more effective ones are the Beetlejail Baitable, Hood Trap, the Freeman Beetle Trap, the West trap, the Australian, AJ's Beetle Eater and the Beetle Blaster [45,46] All these traps have minimal impact upon normal honeybee activity. SHBs present in the hive will be attracted to the trap as a hiding place. Beer or cider vinegar can also be used as bite, as the beetle is attracted by plants and fermentation. Relatively simple and inexpensive, they use non-toxic food grade oil to suffocate the attracted beetles. But only 20% may be trapped, therefore further improvement of the traps is needed [8]. A trap must be installed in every colony in order to have an observable effect [47]. External traps to attract beetles out of the colonies (buckets containing a mixture of honey, pollen and larvae) are also proposed, but with scarce efficacy, since competing with other hives equally attractive [8].

Biological methods are also effective to counter the infestation by *A. tumida*. Pseudoscorpions (*Ellingsenius fulleri* and *E. indicus*) may protect bees from pests such as the small hive beetle [48]. While investigating the use of bottom boards to monitor hive beetles in bee colonies, Torto *et al.* [12] reported that the ant *Pheidole megacephala* was a key predator of larvae at a site in Kenya. Under laboratory conditions entomopathogenic nematodes including *Steinernema riobrave* and *Heterorhabditis indica* have shown excellent control potential against pupating larvae in the ground [49-51]. Soil nematodes specific to the SHB pose no threat to the environment and are exempt from registration and regulation. Beneficial nematodes are used by applying them to the soil while suspended in water. They may be applied as a pressurized spray or simply poured from a watering can. Nematodes applied to soil burrow downward in search of insect pests. Once found, nematodes enter the body of the insect and release symbiotic bacteria, *Photorhabdus luminescens* which quickly kills the pest. Released bacteria dissolve the internal tissues of the insect which becomes food for nematode growth and development. Matured nematodes then mate and lay eggs to produce more nematodes within the dead insect. Several such generations may occur over just a few days. After the inside of an insect is consumed, tiny infective stage nematodes leave the dead insect shell and begin searching for more pests. As many as 350,000 nematodes may emerge from a single dead insect after only 10 days-15 days. Numbers depend on insect size. Furthermore, taking into account that African bees appear to naturally resist to the diseases and pests as *A. tumida* or Varroa mites (*V.*

destructor) that plague nearly all keepers of *A. mellifera* in other parts of the world, selection criteria may be also envisaged.

A more radical approach is represented by the application of eradication measures. The Italian authorities, the Directorate General for animal health and veterinary medicinal products, Ministry of Health, in agreement with the European Commission, developed sanitary measures in accordance with the european legislation, with the support of experts from the National Reference Center for Beekeeping of Experimental Zoo-prophylactic Institute (IZS) of Venezie, Padua, IZS of Mezzogiorno, the Veterinary Task force of the Region Calabria, and in collaboration with the European Union Reference Laboratory for Honey Bee Health, Sophia-Antipolis, France. Protection zones (20 km) and surveillance zones (100 km) have been instituted around infected sites, immediately after the official notification of the first outbreak. All the colonies confirmed contaminated have been destroyed by burning all the hives present in the site, followed by treatments with insecticides of residues and the soil. Teams composed by veterinarians, biologists and beekeeping experts have been deployed to undertake a surveillance program in the field. Monitoring of apiaries, through visual inspection and use of Shäfer type traps in the hives, and soil examination for the search of nymphs of *A. tumida*, have been organized in the protection and surveillance zones and, extended also out of the surveillance zone, on the base of risk assessment [24].

Attention should be paid to the behavioral differences between bees and beetles when burning contaminated colonies. In fact, smoke provokes in bees a well-known reflex pushing them blocked around the comb, avoiding any possible escape from burning hive, while the adults of *A. tumida* flies away at first signs of danger perceiving fire. Therefore, it is necessary to apply methods to prevent dispersion of SHB during operations to destroy hives. Another aspect to be considered is the extension of soil treatment to destroy pupae, taking into account the variability of distance from the hive where larvae migrate to find suitable conditions [8,21]. Furthermore, the adults are not only attracted to bee colonies to reproduce; they can survive and reproduce in other natural environments, feeding on other resources such as fruit, which makes the species very difficult to eradicate [2].

The choice of control methods or the combination of different strategies against *A. tumida* is certainly complex. The results obtained from different approaches followed to achieve the same goal are often difficult to compare, and stress the necessity to improve our understanding of this parasite. The application of control measures demonstrated that it is certainly possible to control its numbers and its impact. Nevertheless, there are opinions that suggest the impossibility to eradicate the new pest [40]. The beetle poses the greatest threat in warm humid climates, but it has also been found as far north as Canada. Much of Europe would therefore seem to offer a suitable habitat for the pest. There is a significant risk that the species could be introduced to other countries in Europe [15]. However, if the Italian authorities succeed to conduct successful eradication measures, this will represent a great result against this dangerous pest, preventing disastrous impact on the EU environment and economy.

Infestations of *A. tumida* can cause direct considerable financial loss to beekeepers [3]. Time and labour to detect and control the beetles and losses in terms of apiaries and honey production and other bee products, are the main economic losses suffered by the beekeeping industry [52]. Within two years of the discovery of *A. tumida* in the USA, at least 20,000 bee colonies had been destroyed by it, costing many millions of dollars. It has had a serious detrimental effect on the beekeeping industry in Australia as well [15]. The infestation by small hive beetles affects the conservation of indigenous bees causing a decline in native bees, such as *A. mellifera*, with negative impact on bee biodiversity [53]. Bee decline will have a significantly negative effect on pollination in habitats where plants rely on bees [54]. Honeybees provide a significant benefit to the environment, pollinating a wide variety of plants [55]. It is likely that honeybees provide over 50% of pollination of naturally occurring plants on which wild birds and mammals depend. Furthermore, infestation indirectly surely affect also food production since bees pollinate more that 30% of the global crop production [56]. In the United States is it estimated that bees pollinate up to $15 billion worth of crops each year [56]. The value of pollination to the UK economy is estimated up to £200 million annually and honey production is worth an additional £10 million-£30 million [57].

A decline in bee numbers was observed worldwide. For example, in UK 100 years ago there were around 1 million bee hives; this had reduced to 400,000 in the 1950s and further reduced to the 274,000 in 2008. The feral honeybee population is reported to have been largely wiped out by disease in the last 15 years [57]. In more than half of European countries-including the UK, France, Germany and Italy, there are not enough honeybees to properly pollinate the crops grown [58]. There may be a number of reasons that have been attributed to explain why honeybees are declining including loss of habitat, poor nutrition, and exposure to pesticides, various diseases and bee pests such as the introduced parasitic mite, *Varroa destructor*, considered to be the most devastating pest of *A. mellifera* worldwide. *A. tumida* comes to add to all other negative factors affecting honeybee health. In conclusion, optimization of control measures, and possibly successful eradication measures against *A. tumida* will contribute to contain the general problem of the decline of *A. mellifera* worldwide.

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