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Appeal for biodiversity protection of native honey bee subspecies of *Apis mellifera* in Italy (San Michele all'Adige declaration)

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Abstract

Honey bees are pollinators that play a key-role in plant biodiversity conservation and crop production. This unique insect species has been managed in hives by beekeepers for millennia, even though such a peculiar animal production system never resulted in the domestication of the western honey bee. The western honey bee was originally distributed throughout most of Europe, Africa, the Middle East, part of the Arabian Peninsula and some parts of Central Asia. From Europe, the honey bee was introduced to America, Asia and Oceania. This adaptation to a range of environmental conditions, together with geological and climatic changes in past eras, has resulted in grouping of *Apis mellifera* into 31 subspecies. In the last 150 years, technological advances in beekeeping and globalisation have heavily endangered conservation of the native subspecies of *A. mellifera* in Europe, with an impact on honey bee production and health status. Evaluation of the impact of this phenomenon on the ecological equilibrium is still ongoing, but there is already scientific evidence of negative effects that this problem is having on beekeeping. This document sets forth the scientific arguments in support of the conservation of native subspecies, and lists the existing Italian legislation in terms of subspecies protection initiatives. It also lists the main factors that are contributing to loss of genetic diversity and of local adaptations. This document does not intend to oppose the actions of the beekeeping industry, but rather to contribute to a more global vision of the very serious problem of honey bee decline.

Key words: honey bee, biodiversity, subspecies, conservation, genetic improvement, beekeeping.

Introduction

The following paper represents the international version of the "San Michele all'Adige declaration" that was drawn up and signed by representatives of authoritative research institutions and by key figures in the beekeeping and environmental fields (note 1). The main purpose of this paper is to make political administrations aware of the urgency of granting adequate protection to the western honey bee (*Apis mellifera* L.) native

subspecies (note 2).

A. mellifera is a species that has been used by humans for beekeeping for thousands of years. Since prehistoric times, wild colonies have been preyed on for honey, brood and wax collection (Crane, 1999). This still happens today for all species of the genus *Apis*, but also for other Apoidea Apiformes (Michener, 2000) in tropical areas (note 3). At all events, the bio-ethological characteristics of the honey bee allowed the development of beekeeping. There is a wealth of iconographic and doc-

umentary evidence regarding this noble human activity, based on archaeological finds dating back to at least 4,500 years ago. For example, among the many decorations found in the Shesepibre Temple in Egypt, built by Nyuserre Ini in around 2,500 BC, there is the oldest representation of a complex and advanced system for management of honey bees and honey, proving beyond doubt the development of beekeeping techniques beginning much earlier (Gritsky, 2015). It is indeed very likely that beekeeping of the honey bee developed around 10-12,000 years ago in the Fertile Crescent, during the era seeing the establishment of agriculture and the rearing and domestication of animals (Bloch *et al.*, 2010; Mazar *et al.*, 2008). Beekeeping has experienced an extraordinary development and diffusion over thousands of years, leading to a wide range of technical solutions, largely still preserved today in different areas of the Mediterranean basin and the Near East (Hatjina *et al.*, 2018). The honey bee and other species of eusocial Apoidea living in complex and permanent societies (like some tropical bees from the genera *Trigona* and *Melipona*) have also inspired a series of symbols, beliefs, and myths, and therefore play an important role in the spiritual, cultural and political evolution of human society at global level.

Despite this very lengthy relationship between honey bees and humans, we can however declare with certainty that this extraordinary animal has never been domesticated.

Indeed, domestication is understood as the process by which an animal or plant species becomes domesticated, namely dependent on cohabitation with man and on his control of feeding and reproduction. Pliny the Elder (Gaius Plinius Secundus, 23-79 AD) had already expressed his opinion that honey bees managed by beekeepers had not been domesticated in the first paragraphs of the book dedicated to honey bees in his '*Naturalis Historia*' (note 4). Likewise, in his work entitled 'Variation of Animals and Plants under Domestication' Charles Robert Darwin (1809-1882) concluded that it was precisely the biological peculiarities of colonies of *A. mellifera* that prevented this process of domestication (Darwin, 1869). Furthermore, Eva Crane (1912-2007), the greatest 20th century expert on apiculture, also provided a clear definition of beekeeping, highlighting the distance from domesticated animals. Indeed, Eva Crane (Crane, 1980) defines beekeeping as "the maintenance of strong healthy colonies of honey bees in hives designed for the convenience of the operator, and the removal from the hives (and subsequent processing) of the products for which the colonies are kept" (note 5). However, the comparison she proposed between beekeeping and the only other similar human activity to it is even more extraordinary: "The use of bees as micromanipulators to harvest food from plants has its nearest parallel in the use of cormorants (on a neck-line which prevents swallowing) to catch fish. The beekeeper has an advantage over the fisherman in that the bees convert the nectar into honey, a very high energy food, before he takes his harvest". Eva Crane refers to the traditional "ukai fishing" with cormorants, practiced in Japan.

It is precisely the fact that the honey bee is not a do-

mesticated animal and retains its wild nature while being managed, that represents the starting point for this document.

Darwin observed that honey bees also behave like wild organisms when they are introduced to areas far from their original area of provenance. Today, when we talk about a wild species and its protection, it is important to establish whether it is a native or exotic organism. The honey bee is native to most of Europe, Africa, the Middle East, most of the Arabian Peninsula and some parts of Central Asia (Ruttner, 1988). It has colonised this extensive area, characterised by a variety of climates and vegetation, over thousands of years, diversifying through natural selection into well characterised populations that have been identified as subspecies, distinguishable firstly on a morphological and ethological basis, and more recently through molecular biology studies (De La Rúa *et al.*, 2005; Meixner *et al.*, 2013). In animal and plant biology, the subspecies is a taxonomic category consisting of one or more populations differentiated from others of the same species by a set of hereditary diagnostic characteristics and originated due to the selective action of various factors and geographical isolation. However, since there are no reproductive barriers between subspecies, if they come into contact, populations can crossbreed and produce fertile offspring. For this reason, no different subspecies can be observed in the same area in nature (O'Brien and Mayr, 1991). It is important to note that when there is no insurmountable physical barrier between two subspecies, they will remain distinct, but in the contact area we can observe the presence of a more or less defined inbreeding zone. Most of the subspecies of *A. mellifera* have areas in contact with one or more different subspecies, but there are also native subspecies on islands and therefore not subject to interbreeding zones. If different subspecies are forced to live together in the same area, due to human activities, they are unavoidably destined to lose their respective unique genetic and phenotypic characteristics (e.g. *A. m. siciliana* and *A. m. ligustica*). To date, 31 subspecies of *A. mellifera* are recognised by the international scientific community (Ruttner, 1988; Hepburn and Radlof, 1998; Engel, 1999; Sheppard and Meixner, 2003; Meixner *et al.*, 2011; Chen *et al.*, 2016).

In Europe and the Caucasus region there are 15 subspecies:

Apis mellifera adami Ruttner 1975 - Crete.

Apis mellifera artemisia Engel 1999 - Russian Steppes.

Apis mellifera carnica Pollmann 1879 - South Eastern Alps, Northern Balkans, Hungary, Slovakia, Rumania.

Apis mellifera caucasia Pollman 1889 - Caucasus.

Apis mellifera cecropia Kiesenwetter 1860 - Central and Southern Greece.

Apis mellifera cypria Pollmann 1879 - Cyprus.

Apis mellifera iberiensis Engel 1999 - Spain, Portugal.

Apis mellifera ligustica Spinola 1806 - Italy.

Apis mellifera macedonica Ruttner 1988 - Northern Greece, Republic of Northern Macedonia, Bulgaria.

Apis mellifera mellifera L. 1758 - Europe norths of the Pyrenees, Alps and Carpathians, to southern Sweden in the north and central Russia in the east.

Apis mellifera remipes Gerstaecker 1862 - Armenia, Azerbaijan.

Apis mellifera ruttneri Sheppard, Arias, Grech et Meixner 1997- Malta.

Apis mellifera siciliana Dalla Torre 1896 - Sicily, Italy.

Apis mellifera sossimai Engel 1999 - Ukraine.

Apis mellifera taurica Alpatov 1935 - Crimea.

In Africa there are a further 11 subspecies:

Apis mellifera adansonii Latreille 1804 - Western Africa ranging from Niger in the north, Senegal to east, and Zambia to south.

Apis mellifera capensis Eschscholtz 1822 - Cape region of South Africa.

Apis mellifera intermissa Buttel-Reepen 1906 - Northern coast of Africa from Morocco at west to Tunisia to east.

Apis mellifera jemenitica Ruttner 1976 - Arid zones of Arabian peninsula, Somalia, Ethiopia, Sudan, Chad, Central African Republic, Niger, Nigeria, Mali, Burkina Faso, Mauritania, Senegal.

Apis mellifera lamarckii Cockerell 1906 - Nile Valley (Egypt and Sudan).

Apis mellifera litorea Smith 1961 - low altitude in

East Africa (Somalia, Kenya, Tanzania, Mozambique)

Apis mellifera monticola Smith, 1961 - Mountains of eastern Africa.

Apis mellifera sahariensis Baldensperger 1932 - Northwestern Africa (Algeria, Morocco) along the southern side of the Atlas range.

Apis mellifera scutellata Lepeletier 1836 - Ranges from South Africa northward along the eastern half of the continent to Somalia.

Apis mellifera simensis Meixner *et al.* 2011 - Ethiopia.

Apis mellifera unicolor Latreille 1804 - Madagascar.

There are a further 5 subspecies in the Middle East and Central Asia:

Apis mellifera anatoliaca Maa 1953 - Anatolia (Turkey and Iraq).

Apis mellifera meda Skorikov 1829 - Iran, northern Iraq and southwest Turkey.

Apis mellifera pomonella Sheppard et Meixner 2003 - Tien Shan mountains and Central Asia.

Apis mellifera sinixinyuan Chen *et al.* 2016 - Xinyuan (Central Asia).

Apis mellifera syriaca Skorikov 1829 - Israel, Jordan, Lebanon and Syria.



Figure 1. Queens and worker bees: **1A)** *A. m. ligustica*, Isola Vicentina, Vicenza, Italy (Photo by Paolo Fontana); **1B)** *A. m. siciliana*, Palermo, Sicily, Italy (Photo by Carlo Amodeo); **1C)** *A. m. mellifera*, Airole, Imperia, Italy (Photo by Fabrizio Zagni); **1D)** *A. m. carnica* × *ligustica*, University of Udine apiary, Udine, Italy (Photo by Giulia Boaro).

In past centuries the honey bee was introduced to the Americas, Oceania and Asia, with the aim of developing beekeeping activities in these regions - activities which can be very profitable with this species. In recent years, the international scientific community has debated whether the massive introduction of honey bees to these regions has had or is having a negative impact on local populations of pollinating insects, especially the Apoidea species, although this seems unlikely according to numerous scientific investigations (Goulson and Sparrow, 2009; Mallinger *et al.*, 2017). However, this issue must be seen within the context of fundamental protection for native pollinator organisms. The status of wild bees in Italy was studied by Quaranta *et al.* (2004).

In Italy, a unique case in Europe, there are natural populations attributable to 4 subspecies. *A. m. ligustica* (figure 1A) and *A. m. siciliana* (figure 1B) are endemic Italian subspecies; *A. m. mellifera* (figure 1C) and *A. m. carnica* (figure 1D), probably present only as populations crossbred to different degrees with *A. m. ligustica*. As regards the original distribution of honey bee subspecies in Italy, as well as Friedrich Ruttner's unsurpassed work 'Biogeography and Taxonomy of Honeybees' (Ruttner, 1988), we can refer to a previous Italian paper published in 1927 by Anita Vecchi, entitled: 'Sulla distribuzione geografica dell'*Apis mellifica ligustica* Spin. in Italia' (Vecchi, 1927). In this paper, Anita Vecchi analysed the chromatic patterns of numerous Italian populations, identifying honey bees with large clear bands in the first abdominal tergites in most of the peninsula, the presence of completely black honey bees in northern Italy and Sicily, and the presence of intermediate colours in certain areas. In the map presented by Anita Vecchi is reported the colour pattern distribution of Italian honey bee (figure 2). This distribution of *A. mellifera* subspecies in Italy, substantially confirmed by Ruttner's study, is well represented by the distribution map published in his above cited text (figure 3).



Figure 3. South-eastern Europe *A. mellifera* subspecies map, from Ruttner (1988).

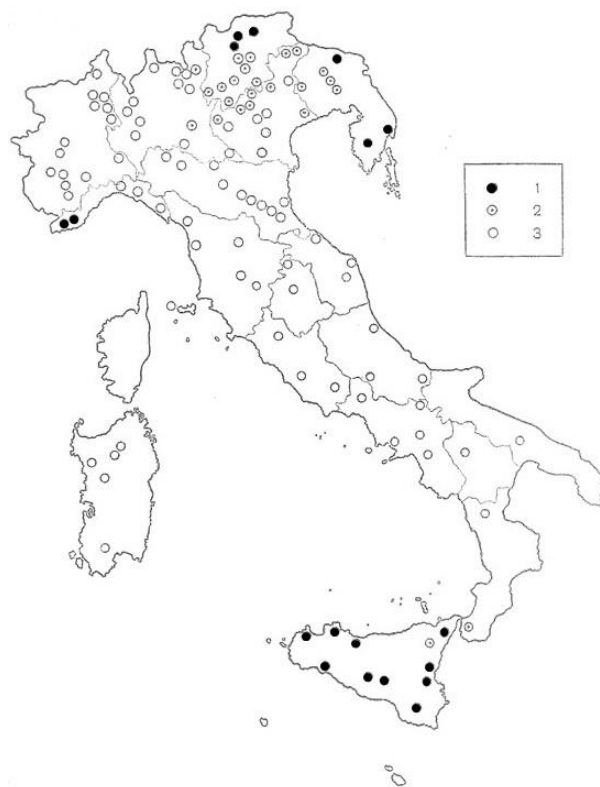


Figure 2. Colour pattern distribution map of Italian honey bees published by Vecchi (1927). (1) locations marked exclusively by the presence of black honey bees, which could represent populations of *A. m. mellifera*, *A. m. carnica* and *A. m. siciliana*, variously crossbred with *A. m. ligustica*; (2) locations where there are populations with intermediate colours; (3) locations where there are only yellow honey bees (typical of *A. m. ligustica*).

The black honey bee, *A. m. mellifera*, also called previously the German honey bee, was present in Italy in the Alps, along the borders with France and Switzerland, in small areas of the Val d'Aosta, Liguria, Piedmont, Lombardy and Trentino Alto Adige Regions, mainly in intermediate form with *A. m. ligustica* (Adam, 1951a; 1951b; Manino and Marletto, 1984). Today, the black honey bee is rare in these regions, but the awareness of the importance of protecting these populations has been growing among beekeepers and institutions, first in France and more recently in Italy. *A. m. carnica* would appear to have been present on the border with Slovenia and Austria, but only in a small part of Friuli Venezia Giulia Region and perhaps in the northern part of the Veneto Region. Today *A. m. carnica*, or rather highly selected strains of this subspecies, and therefore far from having the characteristics of the original populations, are reared by a large number of beekeepers in the Friuli Venezia Giulia Region and Veneto mountains, the Trentino Alto Adige Region, extensive areas of northern Italy and in scattered places throughout the Italian peninsula (note 6).

It is important to remember that the subspecies *A. m. mellifera* and *A. m. carnica* originally had marginal distribution in Italy and in contact with *A. m. ligustica*, so

the limited Italian areas of these two subspecies coincided largely with interbreeding zones (Badino *et al.*, 1982; 1983a; 1983b; 1984; Bolchi *et al.*, 1983; Comparini and Biasiolo, 1991; Gardi and Lodesani, 2004; Leporati *et al.*, 1984; Manino and Marletto, 1984; Marletto *et al.*, 1984a; 1984b; Nazzi, 1992). The whole of the Italian peninsula and Sardinia (although with some particular characteristics, already highlighted by A. Vecchi and more recently in other studies by Floris and Prota (1994) was originally populated by the Italian honey bee *A. m. ligustica*, while Sicily and the surrounding islands were populated only by *A. m. siciliana* (Badino *et al.*, 1985; Manino and Longo, 2010), now known as “the black honey bee of Sicily”.

The subspecies *A. m. ligustica* and *A. m. siciliana* are not only native but also endemic to Italy and their whole original distribution area is included within the Italian territory.

As regards *A. m. ligustica*, it should be emphasised that its distribution over a bioclimatically diverse territory must originally have given rise to many local ecotypes (note 7), each of these well-adapted to particular conditions (Costa *et al.*, 2012), as can also be deduced from studies conducted in Sardinia (Floris and Prota, 1994).

Evolution

A very important aspect of *A. mellifera* subspecies also concerns their origin. All the subspecies have been grouped (Ruttner, 1988) into four lines on a morphological basis: A (Africa), M (Western and Northern Europe), C (Eastern Europe and Asia Minor) and O (the Middle East and Central Asia). The native European *A. mellifera* subspecies belong to three different lines (A, M and C) and differentiated during the last great Ice Age in remote areas in southern Europe (Spain, Italy and the Balkans) and in Africa, from where they recolonized central and northern European regions around 10,000 years ago.

As regards Italy, a study based on nuclear and mitochondrial markers showed that the two Italian endemic subspecies of *A. mellifera* (*A. m. ligustica* and *A. m. siciliana*), originated from hybridization between populations belonging to different evolutionary lines confined within the Italian peninsula and Sicily during the penultimate Ice Age (about 190,000 years ago). In *A. m. ligustica*, attributed to line C on a morphological and nuclear basis, there are also mitotypes of the M line, and in *A. m. siciliana*, which only has mitotypes from the line A, to which it also relates on a morphological basis, it is possible to observe some similarities with line C on a nuclear basis (Franck *et al.*, 2000). This fact, apparently unimportant in terms of conservation, is actually very important because it highlights the complexity, and therefore the fragility, of the structure of European *A. mellifera* populations.

The honey bee has some peculiarities that make it a key organism for conservation of biodiversity and therefore of the global ecological balance.

Honey bees obtain their nourishment from nectar and

pollen (as well as honeydew), and by collecting these substances from flowers provide pollination and therefore reproduction of many plants that require the action of pollinating insects (Porrini *et al.*, 2003). The discovery of the role of insects in the reproduction of many plant species dates back to studies in the 18th century (Sprengel, 1793). Darwin himself studied the benefits of the cross-fertilization of plants and the relationship between certain plant species and the single or few insects capable of pollinating them (Darwin, 1862; 1876). There are several thousand species of pollinators, most of which belonging to Apoidea, a Hymenoptera superfamily differentiated precisely through a process of co-evolution with Magnoliophytes, also called Phanerogams or flowering plants. The genus *Apis* derives from a long evolutionary pathway, and the complex and permanent societies into which the different species are organised play a fundamental role in the conservation of flora in their area of origin. When talking about pollination and pollinators, the tendency is often to consider only the important role that this mechanism has in agricultural production and therefore the direct consequences on human food: considering, for example, that the production of 84% of crop species cultivated in Europe depends directly on insect pollinators, especially bees (Gallai *et al.*, 2009). In fact, since most cultivated plants originate in areas where the main pollinator is the honey bee, this insect effectively plays an extraordinary role in food production worldwide. However, *A. mellifera* has an even greater role in the conservation of spontaneous flora (note 8), namely the plant world underlying almost all terrestrial ecosystems, contributing significantly to the ecosystem services supply. Its ecological plasticity makes this species the main and fundamental pollinator in large parts of the world. One could therefore say that the flora of Europe, Africa, Middle East and restricted areas of Asia has been shaped by the relationship with local populations of this species. Native subspecies of *A. mellifera* are thus fundamental for the conservation of native flora. In other words, honey bees are a typical example of an ecosystem service protecting biodiversity, for which nowadays there is a great awareness. In the last years, numerous scientific studies have reported that *A. mellifera* could act as an invasive species with a major impact on biodiversity, especially in newly colonized areas (Oceania and the Americas) (Moritz *et al.*, 2005; Mallinger *et al.*, 2017). However, although the honey bee has become widespread in nature and has established wild populations in these new continents, the extent to which the introduced honey bees alter biodiversity remains controversial, and there is debate as to whether they have had an effect on the biodiversity of native pollinators, as the most likely group of competing organisms (Goulson and Sparrow, 2009; Mallinger *et al.*, 2017). However, the impact of the movement within the genus *Apis* and of local subspecies of *A. mellifera*, in terms of transporting new parasites or pathogens (Gordon *et al.*, 2014) and of loss of genetic diversity and gene transfer between species has been proven.

In their areas of origin the native subspecies of *A. mellifera* are wild Apoidea! In wildlife terms, protection of the honey bee should be considered in the context of

conserving the natural equilibrium, as well as beekeeping.

Returning to the subspecies of *A. mellifera*, it is clear that being interfertile, these taxonomic entities are in a certain sense fluid and in many cases (neighbouring subspecies) are the result of very precise and refined mechanisms, given by a continuing selective action in terms of climate and vegetation, but also a certain degree of gene exchange with neighbouring subspecies in the interbreeding zones. At the same time, the different subspecies have evolved ethological mechanisms linked to eusociality that have made them more suitable for their habitats, in addition to adapting to the climate and local flora (Alattal and Alghamdi, 2015). Accidental remixing can alter these specific mechanisms for the adaptation of local subspecies to their respective environments. The contact areas between different subspecies allow natural and reciprocal gene exchange, albeit limited, helping to ensure greater potential adaptation to climate change within the subspecies and thus within the species as a whole.

It is therefore essential to repeat that in Italy and in its other areas of origin, *A. mellifera*, even when managed through beekeeping, represents a specific expression of biological information, and should therefore be protected as a component of the wildlife.

Current legislation

Within the framework of the European Union strategy for the protection of biodiversity, The European Parliament recently (1 March 2018) published a resolution on prospects and challenges for the EU apiculture sector (2017/2115(INI)) which in point 20 “underlines the need to preserve the extraordinary genetic heritage, diversity and capacity for adaptation of local, endemic honey bee populations, each tailored over generations to the particularities of their local environment, recalling that this diversity is important in the fight against invasive species, including parasites and diseases”; in point 23 “calls on the Commission and the Member States to put in place measures to increase legal protection and financial support for local honey bee ecotypes and populations throughout the EU, including by means of legally protected locally endemic honey bee conservation areas” and in point 31: “calls on the Member States and the regions to use all means possible to protect local and regional honey bee species (strains of *Apis mellifera* bees) from the undesirable spread of naturalised or invasive alien species having a direct or indirect impact on pollinators; supports the repopulation of hives lost through invasive alien species with bees of local native species; recommends Member States to create centres devoted to the breeding and safeguarding of native bee species; underlines in this regard the importance of developing breeding strategies to increase the frequencies of valuable traits in local honey bee populations; notes the possibilities provided for under Regulation (EU) No 1143/2014 on Invasive Alien Species, as well as potentially under the recently adopted Animal and Plant Health regulations (Regulations (EU) No 2016/429 and

(EU) No 2016/2031 respectively;” (note 9). In Italy, current national, regional and local legislation, of which a broad but not exhaustive review is provided, has a significant number of provisions that involve both a ban on introducing subspecies other than *A. m. ligustica* and local ecotypes in large areas, and more generally, address the issue of protection and incentives for beekeeping. In the European context, an important exception to free trade of animals should be noted in the Republic of Slovenia’s law on the breeding of animals, which defines *A. m. carnica* as a native subspecies and provides for special protection, according to which “the breeding and commerce of reproductive material of other honey bee subspecies is not permitted” in the whole of the national territory (note 10).

Laws prohibiting the introduction of subspecies other than the *Apis mellifera ligustica* and relative penalties

Before proceeding to the citation, by way of example, of some norms and laws issued at national or local level, it is necessary to outline that the texts of these norms and laws have been translated literally even when the terminology adopted is not always correct and sometimes appears confusing. This terminological aspect further highlights the need for a scientific approach also with regard to the rules and laws on the protection of local populations of *A. mellifera*.

Of Italian legislative provisions, the law of 1925 states that: “on the request of the Consortia or beekeepers concerned, or according to a provision of the Italian Ministry of Economic Affairs, Prefects may also prohibit the introduction or diffusion of species, subspecies and strains of honey bees other than *A. m. ligustica* in their respective provinces” (note 11). The Framework Law of 1991 prohibits: “the introduction of alien species, plants or animals, that can alter the natural balance” (note 12). In 2015, an addition to the Criminal Law provided for imprisonment and financial penalties for crimes against the environment: “Anyone who unlawfully compromises or causes a significant and measurable deterioration to the following shall be punished with imprisonment from 2 months to 6 years and with a fine of from 10,000 to 100,000 Euro: 1. Water, air, and extensive or significant portions of the soil or subsoil; 2. An ecosystem, biodiversity of flora or fauna, also agricultural. When the pollution is caused in a protected natural area or area subject to landscape, environmental, historical, artistic, architectural or archaeological protection, or leads to damage to protected animal or plant species, the penalty shall be increased” (note 13). A 2017 resolution adopted by the Chamber of Deputies included not only prohibitions but also protective actions: “(omissis) commits the Government: to take initiatives to safeguard the subspecies *A. m. ligustica*, limiting or banning different subspecies, including hybrids (if not natural), in the Italian territory, through new agreements within the European Union, also implementing a strategy for the protection of biodiversity of this subspecies, providing for sufficiently extensive mating areas (at least 200 square kilometres) in areas where all natural or cultivated hives are inhabited by *A. m. ligustica*” (note 14).

As regards regional laws, two regions provided for ‘buffer zones’ in 1988 and 2009 respectively. The Regional Council of Emilia Romagna: “after consulting the Regional Advisory Committee for Beekeeping, may set up buffer zones around farms, also on the request of a single queen breeder included in the National Registry of Queen Bee Breeders stated in article 12, without prejudice to the application of the current regime of health checks to these. 2. From the moment the buffer zone is established, it is forbidden for third parties to introduce honey bees or increase the number of existing hives” (note 15). Likewise in Tuscany: “Provinces and Mountain Communities (now integrated within the Region) may identify buffer zones around queen bee breeding centres on the basis of specific criteria issued by the Regional Government, concerning the characteristics of the buffer zones, the method for delimiting them and the period during which the ban on the entry of other beehives in the delimited buffer zone is applied, as well as identification of the parties authorised to make such a request” (note 16). In 1992 the Emilia Romagna Region provided for a total ban for the whole regional territory: “it is forbidden to introduce and breed honey bees of strains other than *A. m. ligustica*, as well as interracial hybrids, within the regional territory” (note 17). There are also local bans, such as the 2015 order issued by the Mayor of the Municipality of Vetto (Reggio Emilia) for a delimited area, which states: “that in the territory of the Municipality of Vetto, within a radius of 3 km around Atticola, more clearly indicated on the map, honey bees other than those that are the object of the selection project (Ed. *A. m. ligustica*) may not be introduced or bred” (note 18).

Laws for the protection of *A. m. ligustica*

An Italian law issued in 1992 protects *A. m. ligustica* as a form of wildlife: “wildlife is a public asset of the State and is protected in the interest of the national and international community”. The fact that *A. mellifera* is divided into various indigenous subspecies at local level means that the subspecies, especially if they are endem-



Figure 4. Wild honey bee colony from Morigerati, Salerno, Italy, September 2013 (Photo by Vincenzo Latriglia).

ic, should also be protected as a genuine national heritage (note 19). The 2004 Framework Law on Beekeeping: “recognises beekeeping as an activity of national interest useful for the conservation of the natural environment, the ecosystem and agriculture in general, and is aimed at guaranteeing the natural pollination and biodiversity of honey bee species, with particular reference to the protection of Italian subspecies”, and more specifically at “safeguarding and selection of the Italian honey bee (*A. m. ligustica*) and *A. m. siciliana*, promoting the use of Italian honey bee queens from genetic selection centres” (note 20). Finally, the 2009 ministerial provisions for the implementation of community regulations on organic production establish that: “the choice of the strain to be used in apiculture must favour native subspecies according to their natural geographical distribution: *A. m. ligustica*, *A. m. siciliana* (limited to Sicily) and, limited to border areas, hybrids resulting from free crossing with subspecies from neighbouring countries” (note 21).

Among regional regulations protecting *A. m. ligustica*, there is the 2015 measure by the Autonomous Region of Sardinia: “the Region regulates, protects and promotes beekeeping and encourages the preservation of honey bee species, with particular reference to the Italian honey bee (*A. m. ligustica*) and populations of typical native bees” (note 22).

The Umbria Region’s regulations on beekeeping (note 23), establish in article 93 that: “the Region can set up buffer zones around queen bee producers included in the national register of *A. mellifera* breeders and around mating station situated in the region. Migratory beekeeping is also prohibited in these areas”.

Honey bees, their subspecies and conservation

The seriousness of the situation regarding the preservation of native populations of *A. mellifera* makes it urgent to issue clear ad hoc guidelines focusing exclusively on the problem.

There is also a fundamental aspect to be clarified: for thousands of years, honey bees reared by beekeepers have coexisted with wild colonies of *A. mellifera* naturally present in different areas (figure 4).

Although beekeepers have carried out intense selection activities, especially in the last 150 years, the mating behaviour of queen bees has always guaranteed extensive and beneficial genetic interaction between feral and managed honey bees. With the transfer of the parasitic mite *Varroa destructor* Anderson et Trueman to *A. mellifera*, which will be discussed later, in the last 35 years wild colonies have almost completely disappeared in most of Europe, although there are recent data that could give new insight to this phenomenon (Oleksa *et al.*, 2013; Kohl and Rutschmann, 2018).

This has meant that many debates today on the conservation of the honey bee from the wildlife point of view tend to distinguish colonies present naturally from those managed and selected by beekeepers, from whose swarms they often derive.

Since honey bees are not kept within a fenced and de-

finer area, even when they are managed, safeguarding of *A. mellifera* subspecies cannot consider the protection of colonies present naturally, by now extremely rare, separately from the protection of beehives maintained in the context of beekeeping, from which the feral colonies often derive. Moreover, the protection of each subspecies must be extended to the whole of its original area, because all the local sub-populations (ecotypes) contribute to the conservation and continuous evolution of the subspecies, having adapted to the different habitats in the area. Protecting a subspecies means protecting its variability as extensively as possible. In this context, interbreeding areas with neighbouring subspecies are also fundamental. As regards the conservation of local ecotypes of different *A. mellifera* subspecies, some studies have shown a certain stability of these populations (Costa *et al.*, 2012), as Louveaux summarised for example, asserting that individuals not adapted for natural selection are condemned to die in a short time, meaning that local honey bees are a relatively stable ecotype everywhere (Louveaux, 1969). A recent study involving many *A. mellifera* populations at European level has shown that adaptation by local honey bees makes them able to survive longer in situations of environmental stress and that they tend to produce more honey and be more gentle (Büchler *et al.*, 2014; Hatjina *et al.*, 2014; Uzunov *et al.*, 2014). However, the loss of wild colonies (figure 5) due to *V. destructor* (Potts *et al.*, 2010), the increasing transport of bees outside their relative areas of origin, as well as the increasing use of commercial cross-breed honey bees by beekeepers, poses a great threat to the biodiversity of *A. mellifera* (De la Rua *et al.*, 2009; Meixner *et al.*, 2010) and makes the adoption of restrictive guidelines urgently needed, given that if stabilisation is postponed the situation could soon be no longer recoverable.

An important aspect of biodiversity protection is the economic sustainability of the actions proposed to pursue the objective. In this context, the promotion and differentiation of products deriving from different subspecies of *A. mellifera* could represent an important feature, offering an economic return to the beekeeper with the sale of honey characterised by a specific genetic origin (Utzeri *et al.*, 2018).

We cannot give up on conservation of European subspecies of *A. mellifera* just because they are declining rapidly. This would mean surrendering, condemning to extinction not only these bees, but also the flora they have contributed to shaping (Allen-Wardell *et al.*, 1998). Sooner or later, extinction of European subspecies would also engulf beekeeping in many regions of the world.

The decline of bees

The conservation status of native subspecies of *A. mellifera* in Europe and their respective ecotypes has been seriously compromised.

The causes of this situation can be related to at least six factors.

1) The first, already known since ancient times, albeit to a lesser extent, is the moving of bees (colonies or



Figure 5. Feral honey bee colony in a mesophilic wood on the hills of Isola Vicentina, Vicenza, Italy; September 2013 (Photo by Damiano Fioretto).

queens) from one region of Europe to another by beekeepers (De La Rúa *et al.*, 2009; Meixner *et al.*, 2010). Several subspecies of *A. mellifera* have been involved in this movement (figure 6). There is documentation at least from the 19th century of how certain colonies of subspecies known to be particularly gentle or productive, or even because they are ‘aesthetically pleasing’, such as *A. m. cypria*, have been transferred from their area of origin to different regions of Europe (Canestrini,



Figure 6. Honey bee queen of *A. m. ligustica* ready for shipping (Photo by Cecilia Costa).



Figure 7. Honey bee queen cells obtained with the larvae grafting technique (Photo by Gianfranco Reolon).

1899). The most striking cases, however, concern *A. m. carnica* and *A. m. ligustica*. *A. m. carnica*, has been introduced into vast regions of the natural distribution area of *A. m. mellifera*, in Central and Northern Europe, where it was preferred by beekeepers for its productivity and gentleness to the local *A. m. mellifera*; in the last few decades this subspecies has also become widespread in some parts of Italy, especially in the North-East (Friuli Venezia-Giulia, Trentino Alto-Adige and Veneto regions) (Carpana *et al.*, 2006; Dall'Olio *et al.*, 2007), and according to anecdotal reports by beekeepers also in other parts of the country. *A. m. ligustica*, considered by many beekeeping experts to be the best honey bee for honey production, has spread to many parts of Europe and also to Sicily (where it risked almost completely replacing the local *A. m. siciliana*) but also to the New World, where the black honey bee *A. m. mellifera* was initially introduced (Costa *et al.*, 2015). In Malta, there has recently been some concern regarding the conservation of the local endemic subspecies *A. m. ruttneri*, due to the introduction of *A. m. ligustica* and *A. m. siciliana* (Zammit-Mangion *et al.*, 2017).

2) The second phenomenon that has contributed to compromising the conservation of the native subspecies of *A. mellifera* is the result of techniques for breeding queen bees (Lodesani and Costa, 2003). With the larvae grafting technique, it is possible to rear several thousand queen bees (figure 7) starting from the larvae of a breeder queen characterized by positive characteristics for the beekeeper. In this context, selective pressure to reduce the swarming tendency or the production of drones is detrimental, because it contributes further to the loss of genetic diversity.

Breeding of queen bees on a large scale has on the one hand allowed the selection of highly productive bees for professional beekeeping, but on the other has facilitated the transfer of certain genetic traits of *A. mellifera* out-

side its own area of origin, dramatically increasing the effects of the factor described above (Muñoz *et al.*, 2014). Furthermore, large-scale replication of the genetic heritage of a limited number of individuals today plays a negative role in conserving a large gene pool within the various indigenous sub-species. It is indeed the same specific eusocial structure of honey bees that demands respect and safeguarding of its diversity. The genus *Apis* is indeed characterised by the highest level of polyandry found among social Hymenoptera (Strassmann, 2001). According to most experts, high polyandry, or the mating of queens with numerous males (a phenomenon that leads to high genotypic diversity in the offspring within honey bee colonies) is an evolutionary pathway that the genus *Apis* has pursued (Brown and Schmid-Hempel, 2003; Badino *et al.*, 2004) and which is essential to mitigate the effects of parasites and pathogens on the colonies (Tarpy, 2003). Because of polyandry, the colony of honey bees is made up of a large number of worker bees called 'half-sibs' (with the same mother but different fathers). However, within the colonies there are also a variable number of subgroups of 'super-sisters' (with the same mother and father), equal to the number of drones with which the queen has mated. Because the drone is haploid (the spermatozoa produced by each drone are identical), the super-sister worker bees are individuals with a very high level of kinship, represented on average by 75% genetic similarity.

The presence of super-sister groups is at the basis of the honey bee's social structure, but a reduced presence of these groups can compromise the very survival of the colonies, by reducing the ability to respond to variables such as climate and food resources (Mattila *et al.*, 2012). In an environment with poor genetic variability, a virgin queen on her mating flight will mainly find drones potentially related to each other and to her. Bees seek polyandry, but if during her only mating flight the



Figure 8. Worker honey bees with *V. destructor* mite and with symptoms of deformed wing virus (Photo by Paolo Fontana).

queen encounters only males that are related to each other, following the large-scale reproduction of selected queens, it is as if she had mated with a small number of males and polyandry will not achieve the expected results (Tarpy and Page, 2002).

3) The third negative aspect for the conservation of native subspecies of *A. mellifera* is the adoption of large-scale migratory beekeeping. The spread of motor vehicles in Europe in the 20th century made the transport of whole apiaries from one blooming area to another very simple and rapid, even for journeys covering several hundred kilometres. Thus many northern Italian beekeepers have moved their colonies to the south and vice-versa, mixing up genetically distant populations (ecotypes) of *A. m. ligustica*, but also more recently, transferring *A. m. carnica* to southern Italy, or vice-versa bringing *A. m. ligustica* to interbreeding areas in the Alps.

In many cases long distance honey bee colonies movements take place in the context of so-called pollination services. As migratory beekeeping often takes place during the season in which colonies have many drones and when the mating flights of virgin queen bees occur, the effect of migratory beekeeping is anything but theoretical or negligible.

4) However, the fatal blow to the conservation of native subspecies of *A. mellifera* has resulted from the transfer of the ectoparasitic mite *V. destructor*. It is an obligate ectoparasite of honey bee, originally confined to the Eastern honey bee *A. cerana* that shifted to the new host *A. mellifera* (figure 8) during the first half of the last century. Varroa mites are spread worldwide and is currently considered the major threat for beekeeping industry, due to direct pathogenic effects and indirect effects related to the transmission and activation of viruses during their feeding activity on larvae, pupae and

adults. Being an obligate parasite, the life cycle of varroa mites is closely adapted to that of the honey bee. There are two distinct phases: a phoretic phase when the mite females are on adult bees and a reproductive phase within the capped drone and worker brood cell, in which they complete the cycle by mating with male. The damage caused by varroosis is thought to be a crucial driver for the periodical colony losses everywhere and regular varroa treatments are essential. In fact, lacking of a control program most of the honey bee colonies would collapse within a 2-3 year period. This mite, which like all parasites has co-evolved with the host species in such a way as not to cause irreparable damage to colonies, once transferred to *A. mellifera* as a result of the introduction in Asia of this bee for production purposes has become lethal for colonies (Rosenkranz *et al.*, 2010; Nazzi *et al.*, 2012). Today, varroa mites are one of the main problems for beekeeping in Europe and many other parts of the world, especially where there is very specialized beekeeping. Since the 1960s this parasite has spread rapidly throughout European honey bee colonies, both managed and wild. Until that time, in addition to managed colonies wild honey bees were present everywhere, inevitably crossbreeding with each other, but still subject to natural selection. The presence and abundance of these wild honey bees was essential to limit the negative effects of beekeeping on the conservation of native subspecies and local ecotypes. However, following the accidental arrival of *V. destructor*, there was an almost total disappearance of wild colonies of honey bees in Europe.

This fact, recently confirmed in a study on the conservation of European Apoidea (Nieto *et al.*, 2014) by the IUCN (International Union for Conservation of Nature), was a fatal blow for local populations of *A. mellifera*, to

the extent that today in a way, we are observing the paradox of a situation in which a species fundamental for the conservation of the natural equilibrium, as well as for human nutrition, is surviving in Europe almost only thanks to management by beekeepers.

The presence of subspecies more or less tolerant to varroa in various parts of the world and the discovery of colonies in Europe that can survive the parasite in the absence of chemical treatments (Le Conte *et al.*, 2007; Locke and Fries, 2011), show how, in principle, natural selection can lead to the development of colonies tolerating the parasite starting from local populations adapting to the environment of origin. Furthermore, recent studies comparing various honey bee strains in different European locations have shown that, in general, the colonies best tolerating the parasite tend to be the local ones and that when these are moved away from their environment of origin they lose this important characteristic (Francis *et al.*, 2014; Meixner *et al.*, 2015). These data clearly indicate the usefulness of preserving local populations and the possibility of obtaining colonies tolerant to varroa mites from them, as already attempted in recent research projects at European level.

5) Another relatively recent phenomenon that is threatening the survival of native subspecies of *A. mellifera* is the spread of honey bees selected as 'commercial hybrids' in many parts of Europe and within Italy. These so called 'hybrids' derive from the extensive crossbreeding of different subspecies of *A. mellifera*, also from outside Europe. Distributed on a large scale and widespread among professional and non-professional beekeepers, these bees are further undermining the residual native populations, and as they cannot be reproduced, except by a very few beekeepers and breeders, they represent both a source of genetic 'pollution' and a reduction of the overall gene pool.

These 'hybrids' are not stable and the supposed characteristics for which they are sold are partially related to heterosis (or hybrid vigour) (Lodesani *et al.*, 2009a; 2009b); in subsequent generations the characteristics segregate, with the formation of individuals completely different to each other and mostly with negative characteristics, which can however crossbreed with local populations, preventing beekeepers from implementing selection at local level.

The current lack of protection for native subspecies of *A. mellifera* in Europe also results partly from the fact that at European Union level, with a few exceptions, living organisms are only safeguarded at species level and therefore subspecies are practically ignored. This allows any European beekeeper to request, completely legally, to introduce any subspecies of honey bee coming from other European and non-European Countries, with the sole obligation to follow the veterinary policing obligations.

6) In addition to the previous serious problems contributing to the decline of native subspecies of *A. mellifera* in Europe, at least as regards their biological significance, namely as fundamental components of wildlife and key organisms for the conservation of local flora, and thus overall biodiversity, honey bees, like all the Apoidea superfamily and other pollinating insects, are

seriously threatened by other very serious environmental factors of anthropic origin. These are chemical pollution, especially due to massive and widespread use of pesticides (Porrini *et al.*, 2016; Tosi *et al.*, 2018), environmental changes, with a consequent reduction in nectariferous plants, and climate change. As regards pesticides, in recent years there has been an expansion in the use of chemicals active at very low doses and therefore more complicated to manage from an environmental point of view, whose most serious effects are often at sublethal doses (Matsumoto, 2013; Dively *et al.*, 2015; Doublet *et al.*, 2015; Malagnini *et al.*, 2015; Sgolastra *et al.*, 2017). Extensive use of substances normally considered to be minimally toxic or non-toxic for bees, such as fungicides and herbicides, has instead been shown to be a serious cause of decline for honey bees and Apoidea in general (Pettis *et al.*, 2013; Simon-Delso *et al.*, 2014; 2017; Park *et al.*, 2015; Balbuena *et al.*, 2015; Motta *et al.*, 2018).

All these factors, together with serious genetic deterioration, are jeopardising the survival of local populations of *A. mellifera* and pollinators in general, leading to serious problems for the conservation of flora and thus of habitats. The decline of bees and the impoverishment of flora endanger the survival of beekeeping, which is also of very high cultural significance in historical and social terms, in addition to producing extremely valuable substances for human food and health.

Although the problems are complex, it is necessary to initiate immediate protection actions, based on and supported by concrete scientific data.

The protection of *Apis mellifera*

Many organizations and institutions are working to protect honey bees, and many concrete actions to raise the awareness of political administrations at all levels have been carried out in Italy and Europe and are continuing. Most of these actions, however, are related to beekeeping and are thus based more on animal husbandry than a naturalistic approach. We are aware of the value of these measures to protect bees and raise awareness, but with this document we would like to stimulate public administrations at all levels to put into effect measures designed to protect honey bees and their indigenous subspecies, and in this way to guarantee concrete safeguarding of the environment and apiculture, as demonstrated by the extensive scientific literature.

Future protection strategies should prioritise: (1) creation of a national database on the heritage of *A. mellifera*, on a morphometric and genetic basis, to be linked to the National Honey Bee Register, as a fundamental tool for regulating and managing the heritage, handling and trading of honey bees; (2) boosting of apicultural research to support adequate conservation strategies, encouraging studies aimed at identifying and enhancing local genetic lines and determining the impact of invasive species (plants, animals, parasites and pathogens), integrating this information to understand the potential impact of climate change on the current diversity of bees; (3) promotion of policies aimed at minimizing habitat loss

and making agricultural landscapes 'bee-friendly'.

We therefore wish to strongly urge all administrations and public institutions that can carry out administrative and legislative actions in this context to work urgently to prepare new and concrete measures for the protection of native subspecies of *A. mellifera*.

It is thus a question of safeguarding *A. mellifera* (with the indigenous subspecies and relative local ecotypes) as a species, not in opposition to the selection work carried out by beekeepers, but in harmony with this and according to established principles of conserving bee biodiversity and the related ecosystem services linked to it.

We make this appeal with the conviction that, as regards the two Italian endemic subspecies, protection of *A. m. ligustica* in the peninsula as a whole and Sardinia and protection of *A. m. siciliana* in Sicily, as part of the wildlife and natural heritage, would be not an obstacle to Italian companies breeding queen bees of these subspecies. It would rather make the work of the breeders even more fruitful, as by operating within a protected area they could focus their attention on lines of selection targeted at productivity and the health of the bees used by beekeepers.

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Notes

- Note 1.** The official presentation of the “San Michele all’Adige Declaration” was held in San Michele all’Adige (Trento), Italy on June 12th, 2018 (<https://eventi.fmach.it/Carta-di-San-Michele-all-Adige/La-Carta-di-San-Michele-all-Adige>).
- Note 2.** In Italy the common name of *Apis mellifera* is ‘Ape mellifica’, deriving from the Latin name subsequently proposed by Linnaeus in 1761: *Apis mellifica*. This latter name is not accepted today by the International Commission on Zoological Nomenclature. The second name proposed by Linnaeus in 1761, *Apis mellifica*, means ‘honey bee producing honey’ and would be more correct, while the first, *Apis mellifera*, literally means ‘honey bee carrying honey’.
- Note 3.** For convenience, they will subsequently be described as Apoidea, but refer to Apoidea Apiformes, according to Michener (2000).
- Note 4.** “...cum sint neque mansueti generis neque feri...” or, in English, “...not belonging to domesticated as well to wild animals...”. Pliny the Elder, Naturalis Historia, Liber XI - 4.
- Note 5.** Indeed, Eva Crane uses the verb ‘to keep’ and not ‘to breed’, ‘to raise’ or ‘to rear’.
- Note 6.** In 1927, as is evident on the map in Anita Vecchi’s publication, the Friuli Venezia Giulia Region included a large area now belonging to Croatia and Slovenia, where the *A. m. carnica* lives.
- Note 7.** An ecotype is a separate group of an animal, plant or organism that is closely connected with the environment in which it lives. As such, an ecotype has no taxonomic category.
- Note 8.** As clearly stated in article 1 of Law no. 313 on beekeeping, issued by the Republic of Italy on December 24th, 2004: “This law recognises beekeeping as an activity of national interest useful for the conservation of the natural environment, the ecosystem and agriculture in general and aimed at guaranteeing natural pollination...”.
- Note 9.** Directive 92/43/EEC on the conservation of natural habitats and of wild fauna and flora (Habitats Directive); Communication from the Commission: Our life insurance, our natural capital: an EU Biodiversity Strategy to 2020 (COM (2011) 244); An Action Plan for nature, people and the economy {SWD (2017) 139 final}. The resolution is 2017/2115 (INI).
- Note 10.** Official Gazette of the Republic of Slovenia no. 18/2002, articles 68 and 70.
- Note 11.** Regio Decreto Legge of October 23rd, 1925, no. 2079, “Provvedimenti per la difesa dell’apicoltura”, article 12.
- Note 12.** Italian law of December 6th, 1991, no. 394 “Legge quadro sulle aree protette”. Article 11 - Park regulation, paragraph 3.
- Note 13.** Italian law of May 22nd, 2015, no. 68 “Disposizioni in materia di delitti contro l’ambiente”. Article 1: 1. After Section VI of the second volume of the criminal code the following is included: “Section VI-bis - Crimes against the environment. Article 452-bis. (Environmental pollution).”
- Note 14.** Republic of Italy. Chamber of Deputies, Resolution 7-01250 presented by Zaccagnini Adriano, May 2nd, 2017, no. 787.
- Note 15.** Emilia Romagna Region. Law of August 25th, 1988, no. 35, “Tutela e sviluppo dell’apicoltura”. Article 13, Buffer zone.
- Note 16.** Tuscany Region. Law of April 27th, 2009, no. 21 - “Norme per l’esercizio, la tutela e la valorizzazione dell’apicoltura”. Article 11. Official Bulletin of the Tuscany Region no. 15 of May 6th, 2009.
- Note 17.** Emilia Romagna Region. Decree no. 826 of November 23rd, 1992 of the President of the Emilia Romagna Region “Divieto di introduzione e di allevamento sul territorio regionale di api di razza diversa dall’Apis mellifera ligustica”.
- Note 18.** Provision of the Mayor of Vetto municipality no. 54 of December 18th, 2015.
- Note 19.** Law of February 11th, 1992, no. 157. Article 1. “Norme per la protezione della fauna selvatica omeoterma e per il prelievo venatorio”. Gazzetta Ufficiale Serie Generale n.46 del 25 febbraio 1992 - Suppl. Ordinario n. 41.
- Note 20.** Law of December 24th, 2004, no. 313 - Regulation of beekeeping. Article 1, paragraph 1; article 5, paragraph r.
- Note 21.** Ministerial Decree no. 18354 of November 27th, 2009: “Disposizioni per l’attuazione dei regolamenti (CE) n. 834/2007, n. 889/2008, n. 1235/2008 e successive modifiche riguardanti la produzione biologica e l’etichettatura dei prodotti biologici”. Article 4, Animal production 1) Origin of biological animals in beekeeping - article 8 of Reg. (EC) 889/08.
- Note 22.** Regional Law of July 24th, 2015, no. 19, “Disposizioni in materia di apicoltura”. Article 1, paragraph 2. BUR Region of Sardinia no. 34 of July 30th, 2015.
- Note 23.** Regional Law of April 9th, 2015, no. 12. “Testo unico in materia di agricoltura”. BUR Region of Umbria Supplemento ordinario n. 2 alla Serie generale n. 21 del 15 aprile 2015.