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## Evaluating the use of *Torymus sinensis* against the chestnut gall wasp *Dryocosmus kuriphilus* in the Canton Ticino, Switzerland

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Cover figure: Empty chestnut gall in Stabio, February 2010 (Picture: TULUM SA)

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## Abstract

Following the introduction of the parasitoid *Torymus sinensis* for biological control of the chestnut gall wasp *Dryocosmus kuriphilus* in the Italian Piedmont Region in 2005, and the first record of the presence of the quarantine pest in Switzerland in 2009, a first evaluation of data necessary for a risk assessment of the biological control agent in Switzerland is presented. Information includes present and potential distribution of the gall wasp in Switzerland, its potential economic and ecological damage, possible economic damage reduction by the deployment of *T. sinensis* as well as possible ecological damage/benefit caused by *T. sinensis*. In Ticino, infestation rates of *D. kuriphilus* between 0.82 % and 26.36 % were recorded in eight forests in the Sottoceneri area. The pest will probably spread quickly to all chestnut forests in Ticino, and exceed the 50 % threshold considered necessary for a successful release of *T. sinensis* within a few years. Chestnut forests on the northern side of the Swiss Alps will probably also be infected in the future, as *D. kuriphilus* is already present in the Lake Geneva area, and enough stepping stones consisting in single Chestnut trees or small populations exist to allow natural dispersion across the Swiss Plateau. There are chances that it will colonise central Switzerland from Ticino by human mediated, unintended dispersal on vehicles along the St. Gotthard motorway. Although death of

chestnut forests is not likely due to the sole infection of *D. kuriphilus*, it cannot be fully excluded if combined with other stresses such as drought and chestnut blight. Economic damage may be considerable, particularly for timber and tourist economies. In the case of forest death, damage due to slope instability and disturbance of the water regime may be of particular relevance. The economy of chestnut fruits is still marginal, but big efforts are being undertaken on both sides of the Alps to promote it. Yield losses up to 50–70 % may be expected. Damage to biodiversity may occur if management of chestnut groves is neglected or if old trees die. The release of *T. sinensis* may reduce *D. kuriphilus*' infection below damage levels as demonstrated by Japanese pioneer work. In Europe, however, on a different *Castanea* species, damage reduction has not yet been shown. Although *T. sinensis* successfully established itself in the Piedmont Region, infestation reduction may be visible in the best case in 2010 only. Potential negative environmental impacts associated with the release of *T. sinensis* are poorly studied. These include potential non target effects such as attacks of non-target species and interbreeding with native *Torymus* species (e.g. *T. Affini* or *T. auratus*). An experimental procedure to evaluate these environmental negative impacts of the release of *T. sinensis* is proposed.

# 1. Introduction

The chestnut gall wasp, *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera: Cynipidae) is considered as the most dangerous pest on *Castanea* spp. worldwide (EPPO 2005). It can reduce fruit production and result in tree deaths (Dixon *et al.* 1986). By retarding and reducing photosynthetic area, *D. kuriphilus* infestation reduces shoot growth and biomass production of Japanese *Castanea crenata* Sieb. et Zucc. host trees (Kato and Hijii 1997). Chestnut fruit production may be reduced by 50–70 % (EPPO 2005). Heavy infestation may cause fatal damage to the trees (Moriya *et al.* 2003), particularly by rendering it more vulnerable to other infections such as chestnut blight (Quacchia *et al.* 2008), or coupled to drought (Barthold *et al.* 2004). Where chestnut is planted for timber or to stabilise slopes in Europe, *D. kuriphilus* may have serious economic and ecological impacts (EPPO 2005).

After having been accidentally introduced to Japan, Korea, USA and Nepal, it was recorded for the first time in Europe in 2002, in the Cuneo Province of the Piedmont Region (in northern Italy) (Brussino *et al.* 2002). The introduction probably originated from nursery material introduced from China, 2–3 years before (Quacchia *et al.* 2008). Commerce and transport of nursery material (trees and grafts) are the most common introduction pathways into new countries (EPPO 2005). The pest has since spread to the Lombardy Region, to central and southern Italy, to Slovenia, France (Graziosi and Santi 2008) and Hungary (G. Melika pers. comm.). In 2009, infestations by *D. kuriphilus* were found in southern Switzerland (Forster *et al.* 2009), probably through natural dispersal from nearby Italy. In 2009, it was also found in Maxilly-sur-Léman on the French shores of Lake Geneva, several hundred kilometres from the closest infested area (Département de la Santé des Forêts 2009).

To date no effective plant protection strategies to control this pest are available (EPPO 2005). Pesticides are unsuited and their use often forbidden in forests. Moreover, pesticide treatments would not affect the larvae protected by the gall walls. Pruning is only effective at early stages of infection and before adult emergence. Selection and use of resistant chestnut cultivars against *D. kuriphilus* have been very successful in Japan around 1960. However, by 1970 resistance was overcome by the rapid spread of a tolerant biotype (reviewed in Moriya *et al.* 2003). New resistant Japanese and Korean cultivars have been since selected in the USA (Anagnostakis 1999). The European cultivar Bouche de Bétizac was the first variety discovered to be resistant in the Piedmont Region; several seedlings also showed increased resistance (Colombi 2009). Biological control using the parasitoid *Torymus sinensis* Kamijo has proven to be very efficient in Japan, decreasing *D. kuriphilus* infestation to less than 1 % within a decade after its first release (Moriya *et al.* 2003), thus eliminating

damage on *C. crenata* by the chestnut gall wasp. In the USA *T. sinensis* established itself on galls infecting *C. dentata* (Marsh.) Borkh. (Cooper and Rieske 2007). A release program was initiated in 2005, in northern Italy, on *C. sativa* Mill. (Quacchia *et al.* 2008).

The appearance of *D. kuriphilus* in Canton Ticino has alarmed the competent authorities and the public, due to the high cultural and to a lesser extent economic value of chestnut in southern Switzerland (Colombi 2009). The procedure for the approval of releasing *T. sinensis* in Ticino has been clarified. According to Swiss law, the release of this parasitoid requires a licence (Swiss Confederation, 2008) and the application for the licence needs to include inter alia an evaluation of potential direct and indirect environmental impacts. In fact, the introduction of *T. sinensis* as a biological control agent (BCA) may lead to some environmental hazards. To date, the relation between *T. sinensis* and other gall forming Cynipids, on *Quercus* L. spp. or other Fagaceae is not fully understood. Although some host range tests have been performed before releasing *T. sinensis* in Italy (Quacchia *et al.* 2008), these may not allow to conclusively dismiss the eventuality of non target effects on the environment. Hybridisation with and displacement of a native Torymid species (*Torymus beneficus* Yasumatsu et Kamijo) occurred in the field in Japan (Toda *et al.* 2000, Yara *et al.* 2007) highlighting the possibility of both displacement of native species through competition and genetic erosion. In general, phylogenetic relationships, taxonomic status and biology of parasitoids attacking *D. kuriphilus*, including the individuals released in Europe, are still poorly understood (Aebi *et al.* 2007).

The scope of the present report is to tentatively assess the present and potential impact of the chestnut gall wasp in Switzerland, both economically and ecologically, and to evaluate possible damage reduction and risks associated with the use of the parasitoid *T. sinensis* as a biological control organism. The assessment includes an evaluation of the pest's present distribution and of its infestation rate in southern Switzerland, a prediction of its possible expansion to other areas of the country, a measure of its economic and ecological impact, a literature survey of the use and benefit of *T. sinensis* in Italy and the development of an experimental procedure to evaluate possible hazards associated with the release of *T. sinensis*. Overall, detailed information on the presence of *D. kuriphilus* in Switzerland, its infestation rate, its potential ecological and economic impact and on the effect of the release of *T. sinensis* in Italy would place us for the first time in the position of performing a complete risk assessment which allows for a true cost and benefit analysis for the use of *T. sinensis* against the invasive chestnut gall wasp, threatening chestnuts in several European countries.

## 2. Mission and methods

Infestation rate was recorded on one year old branches as a percentage of infected buds (i.e. buds affected by the presence of a gall) over total amount of buds. Field work took place in southern Switzerland in February 2010 meaning that branches extended during 2008 were harvested for counts. On the buds of these branches *D. kuriphilus* oviposition may have taken place during summer 2008 leading to gall formation in 2009 (Figure 1). Eight sample areas (named A to H) were chosen in the Sottoce-neri area of Ticino for infestation rate recording (Figure 2). In each area, 10 chestnut trees were randomly chosen. For each tree, 10 branches were randomly selected and galls and uninfected buds counted. Recorded data included length of the one year old branch, total number of buds originally produced, amount of infected buds and qualitative differentiation of the gall types. To allow data comparison, the method chosen is identical to the one used by the University of Torino to assess infection rates in northern Italy (A. Quacchia pers. comm.). Single trees were localised using a Garmin GPS™ device and cartographic representations were produced with the Geographic Information Systems software ArcMap 9.3 (license to the Museum of Natural History of Lugano).

A prediction of the invasion corridors to Switzerland, an evaluation of potential economic and ecological damage of *D. kuriphilus*, an evaluation of infestation reduction by the release of *T. sinensis* and a preliminary risk/cost/benefit of the release of *T. sinensis* were assessed through literature surveys, telephone and e-mail interviews or meetings with specialists and internet inquiries (see acknowledgments for detailed information). Meetings were organised with Alberto Alma and Ambra Quacchia from the University of Torino and Giovanni Bosio from the Plant Protection Service of the Piedmont Region in order to gather data on chestnut economy, *D. kuriphilus*' infestation and biological control in Italy. Data on distribution of chestnut on the north side of the Swiss Alps were obtained from Andreas Rudow, Mogli solutions, Baden and from Beat Bäumler of the Centre of the Swiss Network of Floristics (ZDSF/CRSF) in Geneva. Data from ZDSF/CRSF were not used in the present report.

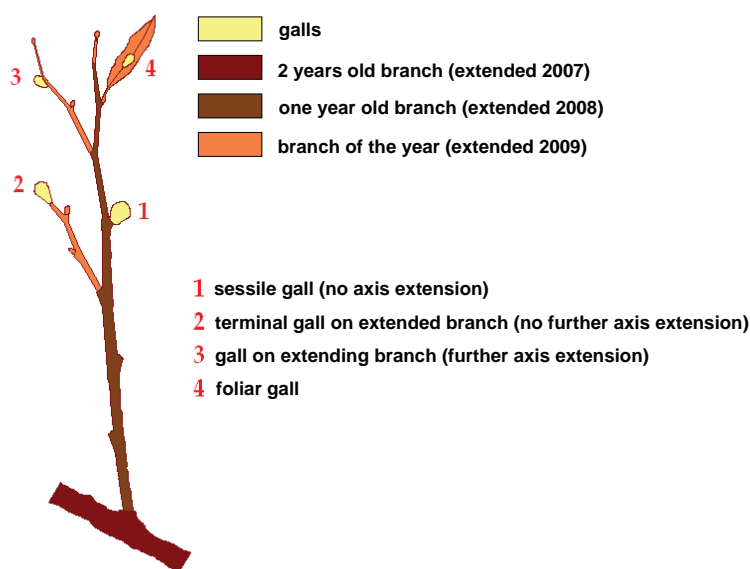


Figure 1. Gall types recorded for infestation rate evaluation



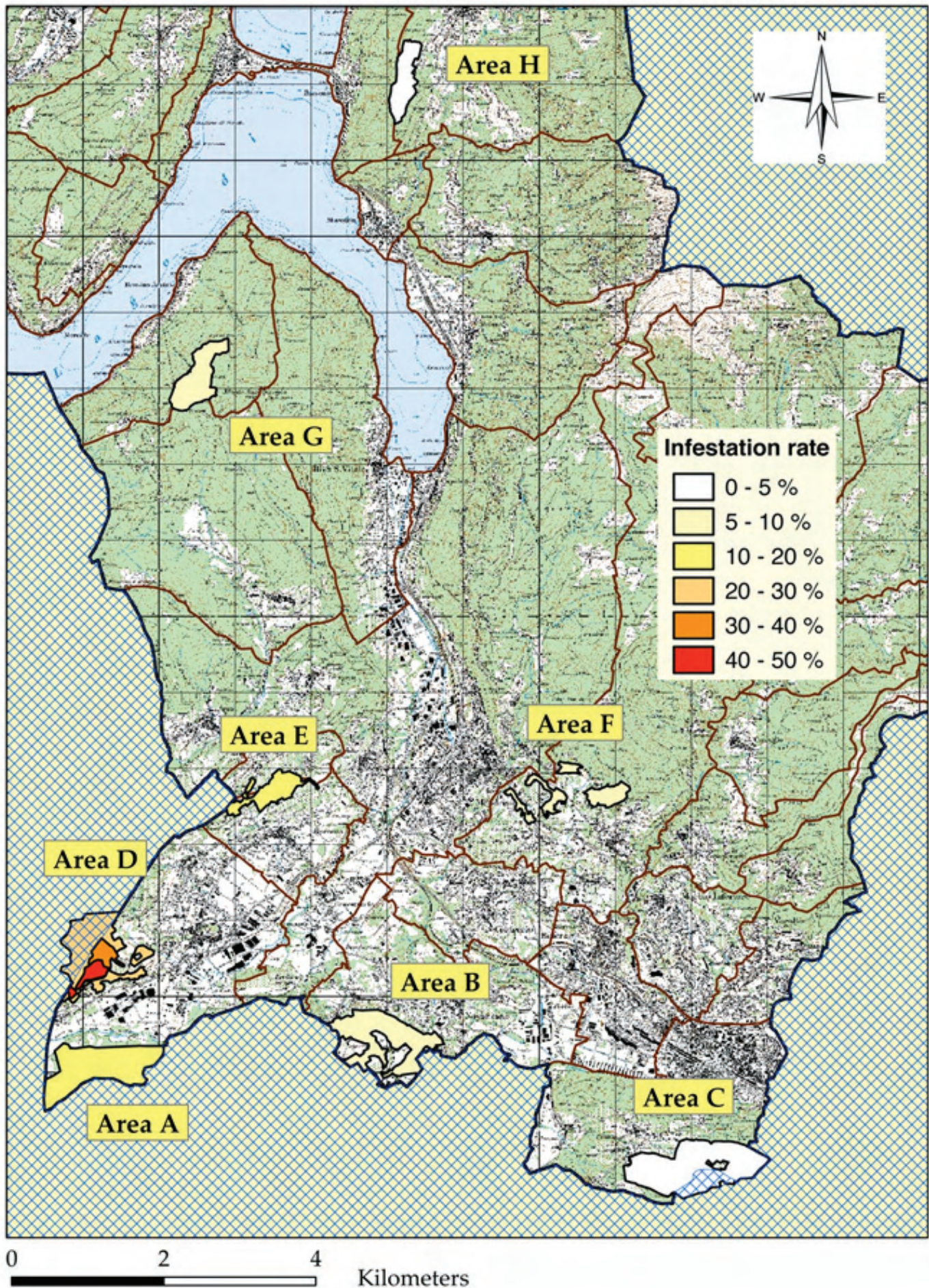


Figure 2. Infestation rates in single areas and probable site of first arrival



### 3. Presence and degree of infestation of *Dryocosmus kuriphilus* in Switzerland

In Switzerland *D. kuriphilus* was first discovered in Southern Canton Ticino in May 2009 (Forster *et al.* 2009). The Cantonal Forestry Office and the Plant Protection Service immediately started a monitoring campaign, concentrating on isolated trees in urban areas, like private gardens and public parks, and chestnut groves in the area of the Mendrisio and Lugano District (Sottoceneri area). Single attacked trees were mapped mainly in the Mendrisio district, the Lugano district being much less invaded (Figure 3). The northernmost attacked tree was found in Taverne, seven km south of Monte Ceneri (which is the geographical barrier between northern and southern Ticino) (Forster *et al.* 2009). We also found *D. kuriphilus* galls in a chestnut grove in Bedigliora (not shown on the map; CH metric coordinates 708806/96001). Low infestation during 2009 was probably very widely spread in all chestnut forests in Sottoceneri, as indicated by the multiple small infestation focuses which can be found with relatively small prospecting efforts. In autumn 2009, *D. kuriphilus* was discovered on old chestnut trees in Castasegna, a village in the Bregaglia valley of the Canton Grisons. It has not been possible to eradicate the infection as the galls were already abandoned (Forster 2009). *Dryocosmus kuriphilus* being classified as a particularly dangerous pest organism in Switzerland (FOAG 2004) and in Europe (EPPO 2005), the Canton Ticino and Grisons issued a decree regulating its handling (Repubblica e Cantone del Ticino 2009, Kanton Graubünden 2009). Notification to the Plant Protection Office of any new infestation focus is mandatory and the commerce with or transport of chestnut plants and grafts originating from infested areas, including buffer zones, is forbidden.

In Ticino, a single infested young tree imported from Italy was found in Grumo (Blenio Valley) and destroyed before emergence of adult *D. kuriphilus* (Forster *et al.* 2009). Several infestation focuses, particularly single chestnut trees in parks and private gardens, were eradicated by the Cantonal Plant Protection Office, the communes and pri-

vate people. However, it is impossible to eradicate *D. kuriphilus* in attacked forests even in the invasion's initial stadiums. This was the case of southern Ticino. Moreover, all chestnut plants present in nurseries in the Sottoceneri area were checked by the authorities including the planting sites of trees sold the previous two years at the Cantonal Forestry Nursery in Lattecaldo (Servizio fitosanitario cantonale 2010). In the area around Stabio, galls formed in 2008 (produced by the offspring of summer 2007) could still be visible in early 2010 indicating that *D. kuriphilus* arrived at least two years earlier than recorded by the authorities.

Based on the cantonal monitoring, eight chestnut forest areas, most of them located on the border between Switzerland and Italy, were chosen for precise infestation recording (Figure 2). Infestation rate is expressed as a percentage of buds developing galls over total buds on one year old twigs. Own surveys showed an overall infestation rate per forest area between 0.82 % (Area H, Arogno) and 26.36 % (Area D, Stabio, overall) (Table 1). Area D (Stabio) being the most heavily infested area, it was subdivided into more homogenous sectors; the red sector is the most heavily attacked with a 40.15 % infestation rate (Appendix 1). On a single tree basis, infestation ranged from 0 % (several trees) to 53.03 % (tree D23, Stabio). A clear gradient in *D. kuriphilus* infestation over the Mendrisio district identifies the forest above Stabio (Area D) as the probable first introduction site in Ticino (Figure 4). The relatively low infestation rates recorded, if compared to values of 80–95 % recorded in the Cuneo Province (A. Alma & A. Quacchia pers. comm.), indicate the recent origin of the infestation, which is still in its initial stadium. Present infestation in Ticino is below the threshold of 50 % necessary for establishment of *T. sinensis*, its release is therefore premature (A. Alma & A. Quacchia pers. comm.). The pest may however spread quickly, and a 50 % infestation rate will probably be reached in the Stabio site (area D) in 2010

Table 1. *D. kuriphilus* infestation rates in 2009 in southern Ticino

Area name	Survey date 2010	Surface (m <sup>2</sup> )	No. of observed trees	Infestation (%)
A – Gaggiolo	1. feb	695221	13	14,37
B – Casate	3. feb	612118	11	5,88
C – Pedrinate	4. feb	922326	10	1,65
D – Stabio overall	6/8. feb	698415	29	26,36
D – Stabio orange	6/8. feb	167845	19	31,63
D – Stabio red	6/8. feb	79037	12	40,15
E – Rancate	10. feb	246082	10	11,44
F – Mendrisio	12. feb	289119	10	7,20
G – Serpiano	25. feb	342773	10	5,46
H – Arogno	25. feb	256514	10	0,82
<b>Total</b>		<b>4062568</b>	<b>103</b>	



already, whereas it can be hypothesised that in the other areas the threshold level will be reached in one or two years time. The rather high infestation rate necessary for a successful establishment of *T. sinensis* in Italy, is intriguing. This species being a specialist of chestnut gall wasp, should, in our opinion, be able to localize its host more

efficiently. The threshold observed by our Italian colleagues may suggest that *T. sinensis* is poorly adapted to *C. sativa* or, that native parasitoids attacking oak and chestnut gall wasps and potentially their parasitoids (acting as facultative hyper-parasitoids) inflict a very high mortality on *T. sinensis* (Aebi *et al.* 2007).

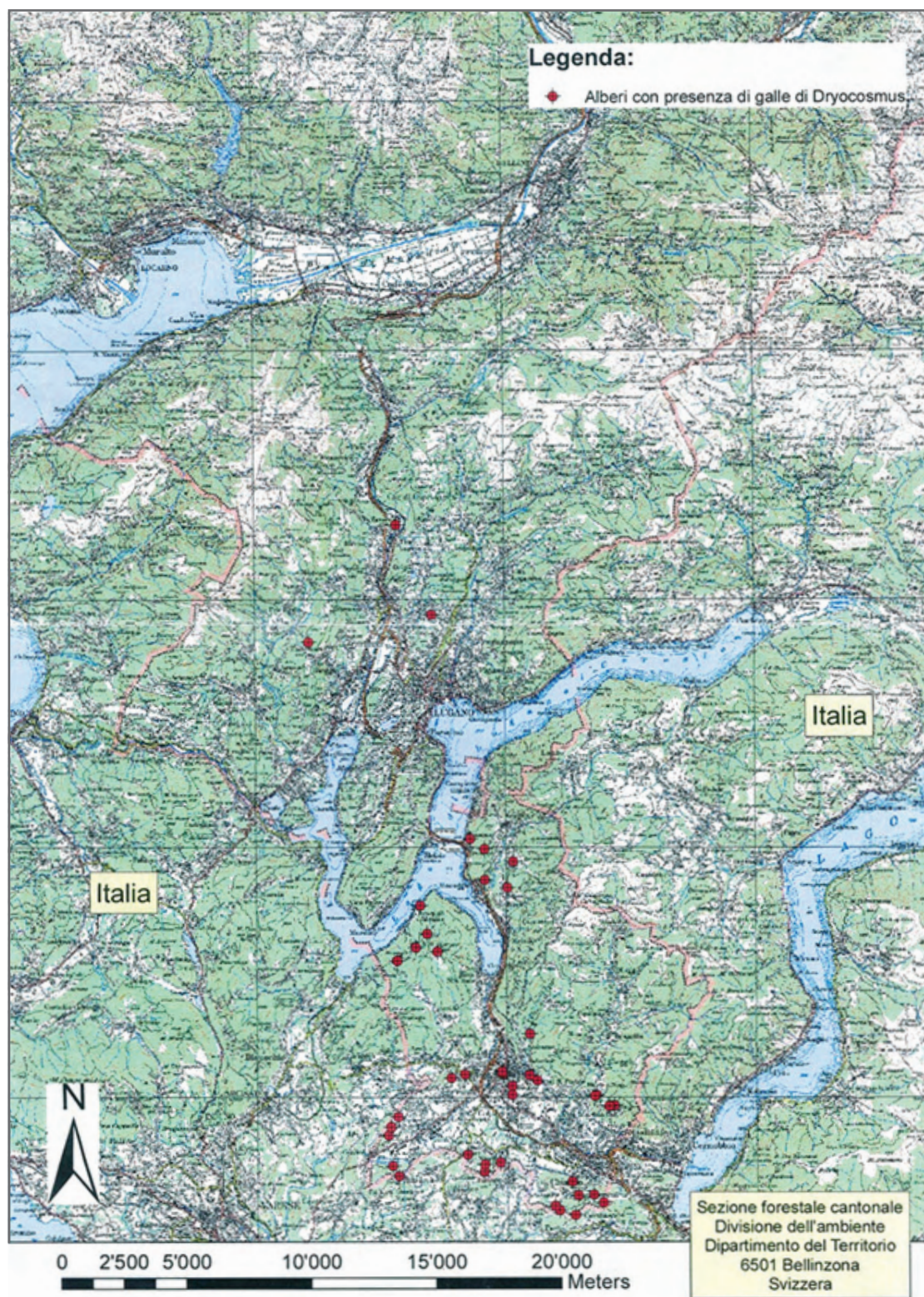


Figure 3. Location of infestation focusses in Ticino in 2009 (© Sezione forestale cantonale)



## 4. Invasion corridors of *Dryocosmus kuriphilus* towards Switzerland

Wind plays a major role in natural dispersion of *D. kuriphilus*. Commerce and transport of infected nursery material represent the major human-mediated pathway of long distance spread of the pest organism (Graziosi and Santi 2008, Forster *et al.* 2009). However, dispersal by the transport of other goods or tourism vehicles should not be underestimated. Road transport may be responsible of the presence and rapid spread of *D. kuriphilus* in the French Roya Valley, Department of Alpes Maritimes, connected to the Cuneo area through the Tende tunnel and pass. In Italy, an infestation focus was discovered at the Madonna del Sasso Sanctuary, a well known tourist attraction on the Orta Lake, on old chestnut trees (G. Bosio, pers. comm.). Another infestation focus was detected in northern Italy in a private garden in a previously pest free area, belonging to a person who drove every day to an infested area near Cuneo for work. These facts strongly suggest that *D. kuriphilus* may spread by unintended transport in cars and trucks. In fact, due to its parthenogenetic reproduction mode, a single female of *D. kuriphilus* may be capable to found a new infestation focus.

Natural dispersion by wind and active flight following first introduction in Italy, accounted for a rapid spread of the pest by 10–20 km each year (reviewed in Graziosi and Santi 2008). Presently, *D. kuriphilus* is considered widespread or almost ubiquitous in the Piedmont region; in the Lombardy region, own surveys showed a widely spread low infection (data not shown). In the USA, the expansion rate was similar, reaching 25 km/year (Rieske 2007). Considering a dispersion over 20 km/year, infestation in the Canton Ticino and Moesa District, where chestnut trees form almost continuous forests on hillsides between 400 and 900 m (Ceschi 2006, Figure 5), may reach the area around Bellinzona and Locarno in 2010, the area around Biasca, the lower part of the Maggia valley and the Moesa valley in 2011, and the upper part of the Maggia valley, the Leventina valley and the Blenio valley by 2012. Thus, within three years all chestnut area in Southern Switzerland may be infected by *D. kuriphilus* to some degree. *Dryocosmus kuriphilus* will also enter Ticino from the Piedmont Region along chestnut forests on the Lake Maggiore. How *D. kuriphilus* will spread to chestnut populations located on the northern side of the Swiss Alps remains more speculative (Figure 5). It

will probably spread from the infested site at Maxilly-sur-Léman on the Geneva lake to the Swiss Chablais region into Valais, and slowly move along the southern slope of the Jura mountains towards central Switzerland. In fact, having been introduced to the north side of the Swiss Alps in the 14<sup>th</sup> century (Rudow *et al.* 2007), *C. sativa* is sporadically present in large areas throughout the Swiss Plateau and northern alpine valleys and no large gaps occur between populations. Figure 6 shows the most recent and complete record of *C. sativa* single stands and areas in northern Switzerland; data relative to Canton Vaud and Geneva are not complete, while data relative to Valais are missing and chestnut area south of the Swiss Alps are not shown (Rudow and Borter 2009). Another invasion corridor towards central and northern Switzerland is represented by the Gotthard motorway (and railway), one of the

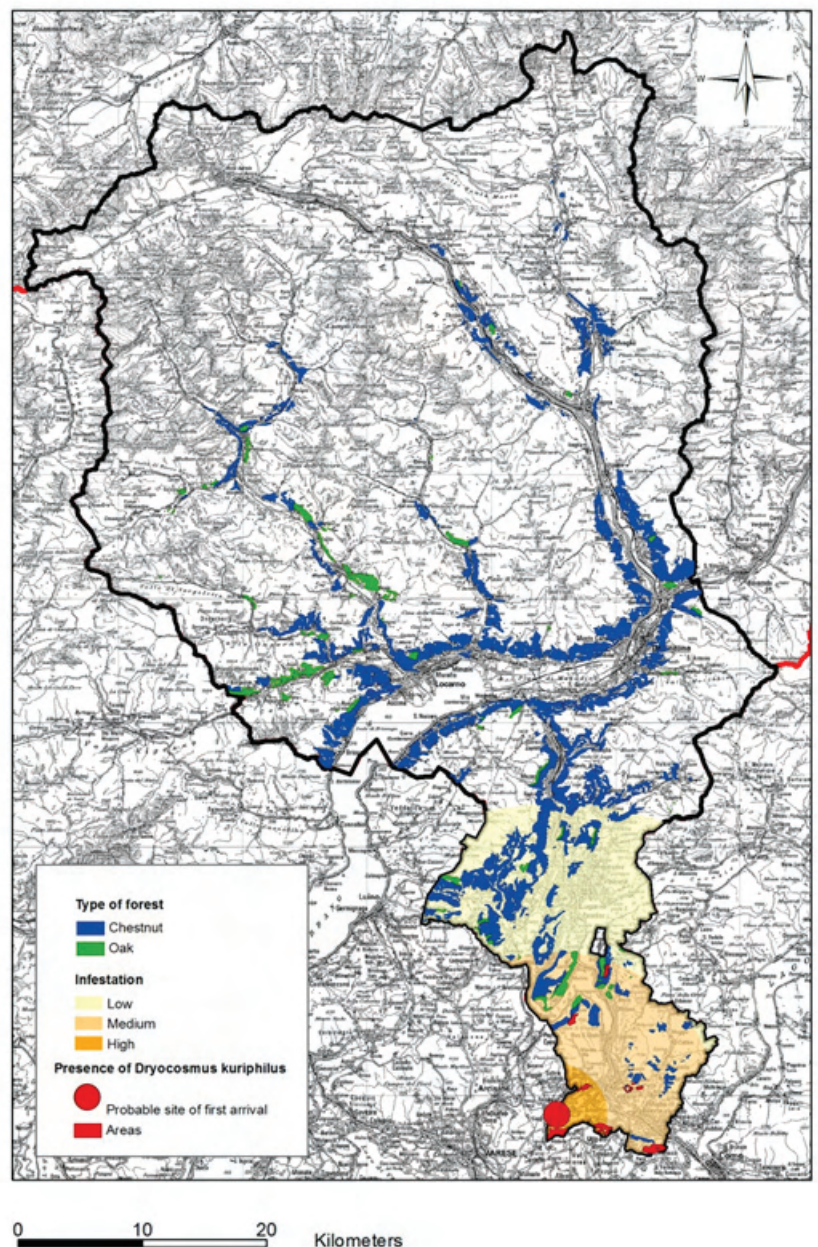


Figure 4. *Castanea sativa* and *Quercus spp.* forests in Ticino (© Sezione forestale cantonale) and infestation in the Sottoceneri



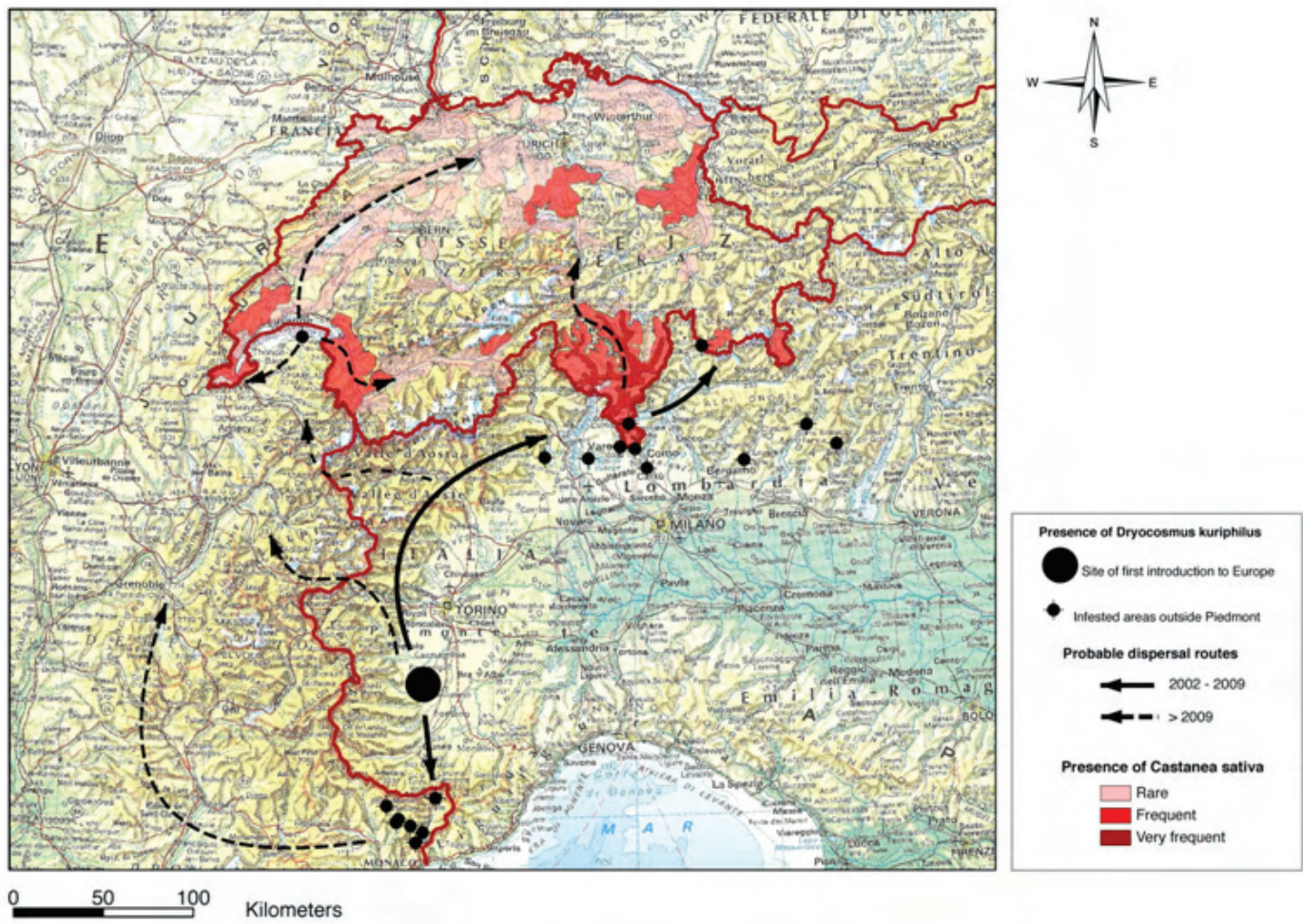


Figure 5. Probable dispersal routes of *Dryocosmus kuriphilus* towards Switzerland

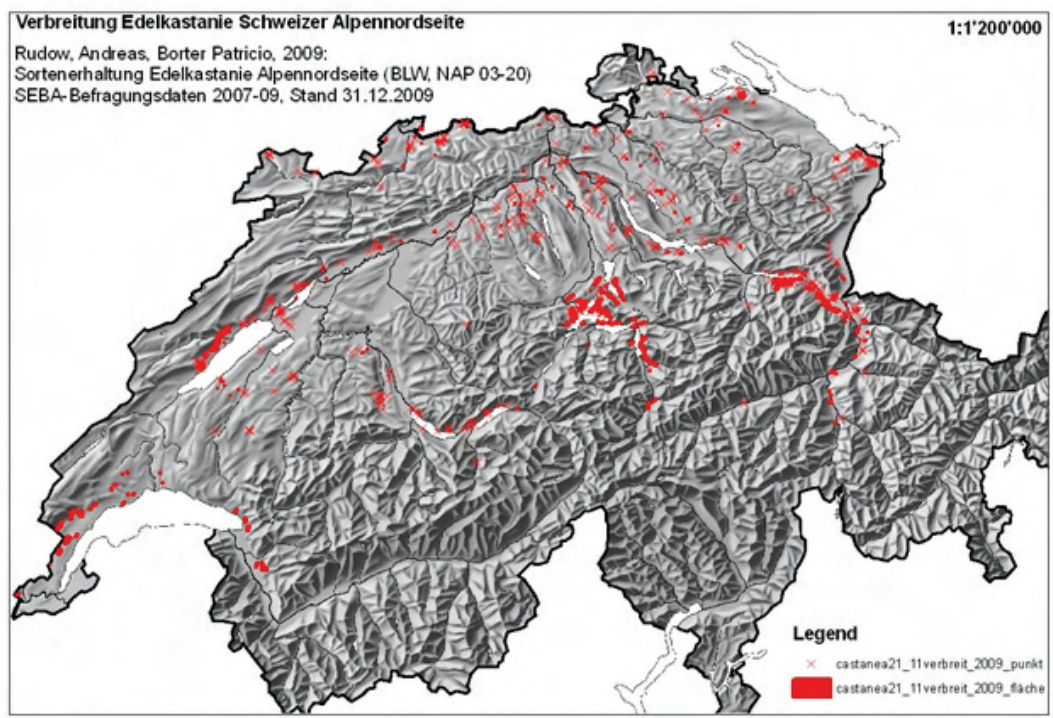


Figure 6. *Castanea sativa* presence on the north side of the Swiss Alps

main north south axis of the whole alpine chain, with over 6 million vehicles transiting each year (Galleria stradale del San Gottardo 2010). There are distinct chances that sooner or later, especially in the case of extensive invasion in Ticino, *D. kuriphilus* will unintentionally reach chestnut stands in central Switzerland by road transport. Human transport may considerably accelerate the spread of *D. kuriphilus* throughout Switzerland. Spread by infected nursery material to northern Switzerland is less probable, as transport of trees from infected areas is forbidden and chestnut groves are mostly professionally managed by people aware of the risk.

*Dryocosmus kuriphilus* will move up north. In October 2009, Jean-Charles Lazareth of the French National Forestry Office, discovered an infestation focus on a recently planted chestnut tree (variety Bouche de Bétizac, which is considered a resistant variety) and on re-growth shoots and lower branches of chestnut trees in a recently restored grove in Maxilly-sur-Léman, on the southern shore of Lake Geneva (J-C. Lazareth pers. comm.), at about 20 km from the Chablais chestnut region. As *D. kuriphilus* wasps already had emerged from the galls before their discovery it was not possible to stop further spread of the infestation in the area. It is presently not possible to pinpoint the exact origin of this infestation focus, the sole in the Rhône-Alpes region located very far from other occurrence areas. To slow down further spread transport of plant material (except fruits) outside the infested area and its buffer zone has been banned (Département de la Santé des Forêts 2009).

Even if not released by Swiss authorities, the biological control agent *T. sinensis* will probably expand from Italy towards Switzerland naturally. In Japan, *T. sinensis* expanded its geographical range at a rate of 1 km/year during the first years. After seven years a steady expansion at a constant rate of about 60 km/year has been observed leading to a natural dispersion of several hundred km in little more than a decade (Moriya et al 2003). Natural dispersion as a function of time (or generations) seems to be linear for *D. kuriphilus* whereas *T. sinensis* increases its dispersal distance exponentially at least in the first years after introduction, meaning that the parasitoid may be able to catch up with the pest. This will probably be the case for the infestation in Ticino, where we can assume that *T. sinensis* will arrive spontaneously in the next 5–8 years, having been released in 2008 in nearby regions in the Italian Regions Piedmont and Lombardy.



## 5. Potential economic and ecological damage caused by *Dryocosmus kuriphilus* in Switzerland

This section attempts to estimate the physical, monetary and non-monetary values of the chestnut, *C. sativa*, in Switzerland. These values need to be gauged before potential economic and ecological damage caused by *D. kuriphilus* can be assessed. In this context, standing volumes, mean annual growth (MAG) rates, unit costs and prices, and conversion efficiencies for timber products have been compiled. When available, the production of non-timber forest products (fruits) was recorded. Non-monetary values such as landscape, culture and recreation as well as ecological considerations for both intact and potentially damaged chestnut forests have also been assessed.

While there is evidence that *C. sativa* may have existed in the region in quaternary refugia for some 8'000 years (Krebs *et al.* 2007), its massive expansion and cultivation in chestnut groves occurred with the Roman domination around 2'000 years ago (Ceschi 2006). Today, 98% of the chestnut area in Switzerland is located on the southern slopes of the Alps, where it covers 19% of the forest area in the Canton of Ticino or 17'000 ha (Ceschi 2006), with 50% of the areas lying below 640 metres above sea level (masl), but with the highest observed elevations reaching 1'200 masl. The map below (figure 7) shows the relative distribution of *C. sativa* in Switzerland.

Over the past 300 years, the cultivation and exploitation of *C. sativa* for timber and non-timber products declined, caused by a variety of contributing factors (Ceschi 2006). One of the more recent factors was the appearance of the chestnut bark cancer (*Cryphonectria parasitica* (Murr.) Barr., which reached southern Ticino in 1948, and parts of Northern Switzerland in 1980. Under European conditions, the disease did not dramatically decimate chestnut stands, but decreased the commercial value of chestnut timber

(Heiniger 1999). Increasing food security of the population, due to the emergence of maize and potato cultivation, and to increased commodity trade, diminished the role of chestnut as a foodstuff even earlier (Ceschi 2006). These developments have led to gradual abandon of both chestnut cultivation for food and forest exploitation for timber and fire wood. They help explaining why chestnut forests in Southern Switzerland are generally over-aged (Fonti and Guidici 2001), and this in turn has its significance for the assessment of potential damage due to *D. kuriphilus*.

FEDERLEGNO, the sector relevant federation of professional associations in the Canton of Ticino, has provided the information contained in Table 2 below (Guidici 2010). According to the 3<sup>rd</sup> National Forest Inventory (NFI 2006), the standing volume of *C. sativa* on the southern slopes of the Swiss Alps was 5.23 million m<sup>3</sup> plus/minus 9%. MAG on this stand was in average 72'000 m<sup>3</sup> between 1995 and 2005. The aggregation of the chestnut timber product categories, as presented in Table 2, results in a MAG value of chestnut forests in Southern Switzerland of CHF 7.5 million. It needs to be reckoned, however, that the current MAG exploitation rate is only about 40 per cent or 30'000 m<sup>3</sup> (Guidici 2010), thus further exacerbating the over-aging of chestnut forests (Fonti and Guidici 2001). This phenomenon is also highlighted by the fact that the southern slopes of the Swiss Alps make up 14% of the national forest cover, but their contribution to the Swiss annual timber harvest of 2008 is less than 2% (BAFU 2009). According to this same source, total timber harvest in Southern Switzerland was 97'000 m<sup>3</sup> in 2008. Thus, the chestnut timber production of 30'000 m<sup>3</sup> per annum is less than one third of the total regional timber production.

FEDERLEGNO has also projected the MAG value of chestnut beyond the forestry feedstock and calculated the output of three representative value chains, i.e. energy chips, poles, and sawn and dried boards. If the total MAG of the considered chestnut forests were converted into these products, an annual value of CHF 24.8 million would result (Guidici 2010). Considering the above mentioned MAG exploitation rate of only 40%,

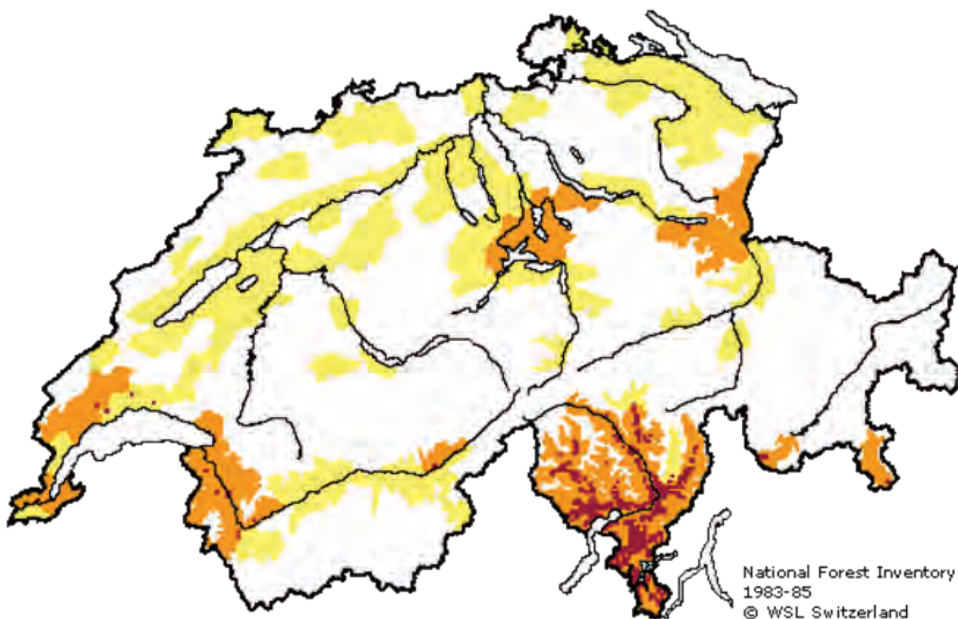


Figure 7. Presence of *Castanea sativa* in Switzerland. Sparse; Frequent; Very frequent (NFI test areas with incidence). Source: <http://www.lfi.ch/resultate/daten/trees/kastanie1.php> (Welten & Sutter 1982)

Feedstock					Value addition			
Wood category	% of total	Available volume (m <sup>3</sup> )	Average unit value (CHF/m <sup>3</sup> )	Total value (CHF)	Value addition to:	Conversion rate from feedstock (%)*	Average unit value (CHF/m <sup>3</sup> )	Total value (CHF)
Energy	60	43'200	55.00	2'376'000	Energy chips	115	112.00	5'564'160
Poles	35	25'200	165.00	4'158'000	Avalanche barriers and playground poles	80	700.00	14'112'000
Boards and planks	5	3'600	275.00	990'000	Sawn and dried boards	40	3'000.00	4'320'000
Conversion residues from poles, boards and planks					Energy chips	20 and 60 respectively	112.00	806'400
<b>Totals:</b>		<b>72'000</b>		<b>7'524'000</b>				<b>24'802'560</b>

\*In the case of energy chips, branches can be included, which are normally not counted in the feedstock volumes. Source: Guidici 2010

the effectively generated added value of the used chestnut wood categories is in the range of CHF 10 million per annum.

Non-timber uses of chestnut included the extraction of tannins for leather tanning or color pigment production, which has been halted about fifty years ago, and the collection of chestnut fruits. The latter still has economic importance in the Piedmont Region of Italy (with 2'500 to 4'000 metric tons per annum) while the chestnut production in the Canton Ticino has oscillated between 8 and 56 metric tons in the years 2001 to 2008 (P. Bassetti pers. comm.). The same source estimates that sales of fresh chestnuts and chestnut flour, assuming an annual harvest of 50 metric tons, would be in the order of CHF 180'000 at wholesale and CHF 250'000 at retail prices.

With the ongoing rehabilitation programmes of chestnut groves (*selve*), both South and North of the Swiss Alps, the domestic production of chestnut fruits and derivatives is expected to increase. One programme in Central Switzerland (Pro Kastanie Zentralschweiz 2008) estimates to reach an annual output of 50 metric tons of chestnut fruits before 2018, and 200 metric tons by 2050, from an aggregated grove area of 38 ha. CHF 800'000 have been invested into this program, and a second tranche of another CHF 800'000 is likely in 2012 (Rudow 2010). Private grove owners, sponsors, cantonal administration and the Swiss Landscape Fund participate in this venture. In the Canton Valais, the Municipality of Fully organises each year a chestnut festival, attracting 270 exhibitors and 40'000 visitors over two days, with 7 metric tons of chestnut fruit sold. In the neighbouring Canton Vaud, a chestnut growers' association in the Chablais region has been set up that sells collected chestnut from groves that are again rehabilitated and maintained, reportedly to an extent similar to the Valais (Masson *et al.* 2010).

For the Canton Ticino, the public investments for chestnut grove rehabilitation are shown in Table 3. In 2008, the aggregate federal, cantonal and municipal contributions for the forestry sector in the territory of Ticino amounted to CHF 3.9 million (BAFU 2009). Thus, chestnut grove rehabilitation was assigned slightly more than 20% of the available funding for the forestry sector that year. Other sources confirm that this was and is part of an official rehabilitation strategy (Moretti 2006a).

**Table 3. Canton Ticino – Public investments in chestnut grove rehabilitations, 2004–2009**

Years	Annual budgets (CHF)	Area (ha)	Average budget per ha (CHF)
2004	180'000	3.3	54'545
2005	404'000	7.3	55'342
2006	445'000	8.1	54'938
2007	633'000	11.5	55'043
2008	810'000	13.5	60'000
2009	660'000	12.9	51'163

Source: Cantonal Office of Forestry, Bellinzona, 2010

The various tourism promotion offices of the Canton Ticino have opened several footpath itineraries dedicated entirely to chestnut and its historical and cultural connotations (Ticino-tourism 2009). Other initiatives, such as guided tours and chestnut markets, have gained considerable visibility, among the local population as well with tourists (Società commercianti Bellinzona 2007).

In view of the above, it is fair to say that chestnut is presently in the midst of what can be called a revival in Switzerland. It is difficult to say, without a comprehensive survey, what kind of motivations are behind this phenome-

non. Intact chestnut forests that build a more or less contiguous seam on the fringes of the main valleys of Southern Switzerland, with a concentration on the generally steep parts of the valley flanks (see Figure 5), have obvious ecological advantages, such as fixing the soil cover and maintaining an even water supply regime. There are, to our knowledge, no studies available that assess such an environmental value specifically with regards to chestnut. Chestnut forests, especially abandoned coppice woods as they are frequent in Ticino, are rather poor in species diversity, and of feeble conservation value for biodiversity. Managed chestnut groves, however, particularly with the presence of old trees, offer diversified structures to a specialised fauna. They provide a habitat to several rare bird species such as *Ficedula albicollis* Temminck which is still present in Ticino, several bat species (all protected) including the threatened *Nyctalus leisleri* Kuhl, for which chestnut groves in Ticino have a very high conservation value (Zambelli *et al.* 2008), and several rare insect species. Managed chestnut groves are generally much more bio diverse than abandoned groves, with twice as many bat species and 1.5 times more invertebrates (Moretti 2006b).

It can also be argued that intact chestnut forests and a growing number of well maintained and exploited chestnut groves are one of the building blocks for successful tourism operations. According to generally accepted estimates, tourism in Ticino contributes 12 % to the regional gross domestic product (GDP), which was in the order of CHF 20 billion in 2008 (RSI 2010). The GDP contribution of tourism is however controversial to some extent because the economic statistics of the Canton Ticino do not avail of a specific satellite account for tourism as this is the case for other cantons and the Swiss Confederation. Some authors estimate the total contribution of tourism to the regional GDP at over 20 % (Emma 2009), others at below 10 % (RSI 2010). For the sake of this work, the figure of 12 % of CHF 20 billion is assumed. Let's assume that the invasion of *D. kuriphilus* and the ensuing destruction of chestnut forests and groves would depress tourism sector GDP in Ticino by 1 % and then 5 %, respectively. At 1 % decrease of tourism GDP per annum, the damage would already be equivalent to the potential annual timber value calculated in Table 2, i.e. CHF 24 million. At 5 % slump would be equivalent to CHF 120 million. These figures infer that *D. kuriphilus* indeed has a considerable damage potential.

In this context, it is necessary to have an idea whether *D. kuriphilus* would indeed be in a position to inflict tangible damage to chestnut forests. The example of the chestnut cancer (*Cryphonectria parasitica*) in North America on *Castanea dentata* is ominous in this respect. On 11<sup>th</sup> April 1911, the New York Times predicted that "blight is killing chestnut trees... disease is like a cancer" (The New York Times 1911). Indeed, within 40 years, the near-4-billion-strong American Chestnut population in Northern Ame-

rica was devastated – only a few clumps of trees remained in California and the Pacific Northwest (Wikipedia 2010). According to Japanese researcher (Kato and Hijii 1997), *D. kuriphilus* does normally not kill the chestnut tree, but tree development and fruit production are compromised. It is not known what effect the concurrence of several stress factors, such as a massive *D. kuriphilus* infestation on chestnut populations weakened by the chestnut cancer (which is the case in Southern Switzerland), and moreover in a year of drought, would cause in terms of mortality or stunted growth. The drought year of 2003 alone was sufficient to cause spontaneous mortality in chestnut forests (Barthold *et al.* 2004). Consequently, the probability of *D. kuriphilus* of becoming a major source of economic and ecological damage in Ticino may be relatively high, and possibly within a few years.

Comparing relevant magnitudes of the Italian chestnut economy with the ones of Southern Switzerland is difficult because available Italian references emphasise chestnut fruit production and not timber related aspects as is the case with the Canton Ticino. Out of 893'000 ha of forests in the Piedmont Region, 205'000 ha comprised chestnut in the year 2000, and chestnut groves were estimated to cover more than 9'000 ha in the same region. In 2009, the Italian chestnut fruit production was 45'000 metric tons, to which Piedmont normally contributes about 10 %. Italy occupies the fourth rank of chestnut producers in the world after China, South Korea and Turkey (Regione Piemonte 2009). 2007 was the worst ever chestnut year in Piedmont with 2'500 metric tons, a slump attributed to drought and to *D. kuriphilus*. After 2007 chestnut harvests in the Piedmont Region increased again. The recovery is not correlated with the presence of the biological control organism considered the very low parasitism rates until last year. The last two years were extraordinary rainy, leading to longer shoots and thus more buds generated after *D. kuriphilus* oviposition, leading to healthy shoots and flowers the following year (G. Bosio pers. comm.). Factors other than parasitism can strongly influence chestnut productivity respectively *D. kuriphilus* damage.

Some 3'400 chestnut farming units with an average of 1.7 ha in the region were affected by this development (Regione Piemonte 2009). The appearance of *D. kuriphilus* has alarmed the competent authorities and the concerned public considerably, not only for economic short term reasons but also for the fear that the chestnut gall wasp could spell the practical disappearance of *C. sativa* in the region after a continuous decline in chestnut grove area of over 50 % between 1982 and 2000. Particularly badly hit by *D. kuriphilus* would be Cuneo Province where 87 % of the Piedmont Region chestnut grove area is found (Regione Piemonte 2009) and where chestnut is not only found in groves but also in mixed forest stands.

## 6. Release of the parasitoid *Torymus sinensis* in the Piedmont Region, Italy

Confronted to the chestnut gall wasp invasion and to the lack of alternative control strategies, the Plant Protection Service of Regione Piemonte and the University of Torino, started a releasing campaign of *T. sinensis* in 2005. Releases were performed in three sites with high chestnut gall infestation and conditions facilitating natural dispersal of the parasitoid (Quacchia *et al.* 2008), such as hill tops and areas with continuous chestnut presence. Chestnut galls were imported from Japan in 2005 and 2006, and *T. sinensis* emergence synchronised with *D. kuriphilus* gall formation in Piedmont by delaying larval development at low temperatures. By 2006 *T. sinensis* had been released in 11 sites, one of which was chosen as a rearing site for further mass production of the parasitoid. A total amount of 2117 *T. sinensis* individuals (1148 females and 969 males), was released between 2005 and 2006, i.e. about a hundred females per site (Quacchia *et al.* 2008). The release of hundred couples (100 females and 100 males) is considered to be adequate to establish a viable and spreading population of *T. sinensis* in a given site, provided *D. kuriphilus* infestation rate exceeds 50% (A. Alma & A. Quacchia pers. comm.). In 2007, further 4000 galls were imported from Japan (Colombi 2009) and more couples of *T. sinensis* were introduced in the first three sites of release, summing up to 200 couples released over three years (2005–2007; A. Alma & A. Quacchia pers. comm.). Based on the gathered experience and the production of *T. sinensis* in the rearing site, *T. sinensis* has been released in 84 sites in the Cuneo Province (covering almost its entire chestnut production area), 3 sites in the Torino Province and one site in the Verbano-Cusio-Ossola Province (Madonna del Sasso), located about 40 km beeline from the Southern tip of Ticino no later than 2009. Outside the Piedmont Region, the biological control agent has been introduced in the Italian Regions Lombardy, Liguria and Latium in 2008 (Colombi 2009).

Settlement prospects were assessed by collecting galls and checking *T. sinensis* emergence in the first three release sites and the rearing areas in winter 2006/2007. In the release sites, *T. sinensis* emerged from about 0.08% of the galls whereas in the rearing site, per gall parasitism was 0.53% (Quacchia *et al.* 2008). To assess infestation reduction and eventual damage reduction, it has to be considered that one gall may contain between 1 and 35 *D. kuriphilus* larvae with an average of 3.5 larvae/gall. Gall parasitism averaged about 1% in 2008, increasing up to 17–23% parasitized galls by 2009 in these sites (A. Alma & A. Quacchia pers. comm.). Thus, a reduction of *D. kuriphilus* infestation by the parasitoid may be measurable only during the 2010 season in the areas of first release of *T. sinensis* in Europe. However, research conducted at the University of Turin indicates that *T. sinensis* can form stable populations in the wild.



## 7. Potential benefits and damage due to the release of *Torymus sinensis*

Biological control using *T. sinensis* was successfully implemented in late 1970 and early 1980 in Japan to fight against *D. kuriphilus* on *Castanea crenata* (Moriya *et al.* 2003). The parasitoid settled successfully, and within seven years after first release the number of females emerging per 100 galls increased by 25 times (Moriya *et al.* 2003). The infestation rate on *C. crenata* decreased from 43 % to 3 % within six years after release of *T. sinensis*. Ten years after release it had decreased to less than 1 %, much lower than the tolerable injury level of 30 %. In the USA, *T. sinensis* established itself on galls infecting *C. dentata* (Cooper and Rieske 2007), whereas in northern Italy a release program started in 2005 on *C. sativa* (Quacchia *et al.* 2008). A reduction of chestnut gall wasp infestation and possible damage reduction cannot be assessed in Europe yet; first relevant data may be generated starting from the 2010 season. In fact, 80–95 % infestation rate is still recorded in the Cuneo Province (A. Alma & A. Quacchia pers. comm.). According to the University of Turin and the Plant Protection Office of the Piedmont Region, a monitoring of 10–15 years is necessary to assess effects of the introduction of *T. sinensis* with confidence.

Classical biological control of insect species has been successfully used for more than 120 years. The release of more than 2000 species of natural enemies has resulted in the control of at least 165 pest species worldwide. While safety of biological control was generally not questioned until the beginning of the 1990s, an ongoing debate started shortly after agreement of the Rio Convention on Biodiversity in 1992. Since then, international organizations and national governments published general principles of guidance for the import and the release of invertebrate biological control agents. Only recently a methodological guide on how to measure the environmental impact of invertebrate for biological control of arthropods was published as a result of the European project REBECA (Bigler *et al.* 2006, <http://www.rebeca-net.de/>). Risks and benefits of releasing a beneficial insect should always be weighed against each other prior to its introduction in a new environment. A comprehensive environmental risk assessment relies on the identification and evaluation of potential risk of releasing a natural enemy and on a plan to minimize risk and mitigate unwanted effects of the biological control agent. The final step in the decision-making process of whether or not to introduce and release an organism in a new environment is to identify, assess and weigh up all adverse and beneficial effects in a risk-cost-benefit assessment. Diverse aspects such as expected costs and benefits on economic values, human and animal health, the environment, as well as social and ethical aspects are considered.

The use of *T. sinensis* as an antagonist is probably today's most efficient and safe control method against *D. kuriphilus*. Nevertheless, biological hazards and unforeseen side effects derived from the introduction of a new species need to be carefully assessed. In Japan unwanted effects associated with the release of *T. sinensis* were observed. For example, hybridisation between *T. sinensis* and *Torymus beneficus* Yasumatsu et Kamijo, a native Torymid, has been observed in the field (Toda *et al.* 2000). In addition, displacement of the native species by the introduced one has also been observed in the field even if its causative agent has not been formally identified (Yara *et al.* 2007).

We identified two kinds of potential unwanted non-target effects as potential consequences of a *T. sinensis* release in Ticino. First, the host-specificity of *T. sinensis* should be tested with a particular attention to native oak gall wasp species (Hymenoptera: Cynipidae). Second, potential hybridization and interbreeding among *T. sinensis* and native *Torymus* species should be clarified. These conclusions about the potential environmental risk associated with this parasitoid are shared with the EFSA Panel on plant health established (EFSA Panel on Plant health 2010). An experimental procedure to evaluate the identified potential risks is proposed in the following two sections.



## 8. Host-specificity testing for *Torymus sinensis*, a parasitoid of the chestnut gall wasp *Dryocosmus kuriphilus*

Host-specificity testing for the use of natural enemies of insects to achieve biological control only gained importance in the last decade. Only 1.5% of biological control agents (BCA) used before 1999 have been through a host-specificity evaluation (Lynch *et al.* 2001). Selecting the appropriate species for testing potential detrimental impact of the BCA on the environment is paramount to a sound environmental risk assessment. The centrifugal phylogenetic method of Wapshere (1974) is commonly accepted as the first appropriate method to list potentially endangered species even if other factors such as the feeding niche or the natural habitat of the BCA and potential preys or hosts should be considered (Kuhlmann *et al.* 2006). A major limitation to the full description of a parasitoid's host-range is imposed by their biology as parasitoids must be reared to adult stage on several hosts to assess the suitability of the latter for a given species.

Despite this difficulty, information on ecological determinants such as host habitat, host-location strategies, physiological interactions with hosts and host phylogeny may be useful to evaluate a parasitoid's host-range (Stireman and Singer 2003). In general, host taxonomy and shared ecology are regarded as the most relevant factors influencing host-range (Askew and Shaw 1986). Indeed i) parasitoid may attack closely related hosts sharing similar physiological attributes and defense mechanisms and ii) closely related parasitoids may attack hosts feeding on similar host plants or with similar feeding niches (Kuhlmann *et al.* 2006).

The parasitoid *T. sinensis* is believed to be host specific but its host-range was never appropriately tested in its native nor in its introduced ranges (EFSA Panel on Plant health 2010, Murakami *et al.* 1977; Cooper and Rieske 2009; Stone *et al.* 2002; Zhang 2009). The only experimental assay was undertaken by Quacchia and colleagues in 2008 and involved galls of *Mikiola fagi* (Diptera: Cecidomyiidae), *Cynips quercusfolii* (Hymenoptera: Cynipidae) and *Andricus kollari* (Hymenoptera: Cynipidae) which were offered to female *T. sinensis*. In these experiments, the absence of behaviors such as host encounter, host inspection and host attack lead to the conclusion that no direct effects on these non-target species were expected. A recent study highlighted the following problems in the species selected to evaluate the host-range of *T. sinensis* in Italy (EFSA

Panel on Plant health 2010): i) the phylogenetic distance between target and tested non-target species and ii) a difference in phenology between target and tested non-target species at the time when female *T. sinensis* are released in the field. There are major doubts about the choice of *M. fagi*, *A. kollari* and *C. quercusfolii* as potential non target organisms. In spring, when the tests have been performed in Turin, the galls of *M. fagi* were already mature. Moreover, the species is phylogenetically very far from gall wasps, and quite surely non attractive even to generalist *Torymus* species. Similarly, *A. kollari* and *C. quercusfolii* galls do not harbour developing larvae in spring, adults developing during the previous seasons. Based on the experiments, it therefore cannot be concluded that *T. sinensis* will not switch onto native gall makers. Following the recommendations of Kuhlmann and colleagues (2006) the EFSA Panel on plant health established a new species list for host-specificity testing for the gall wasp parasitoid *Torymus sinensis* (table 4) (EFSA Panel on Plant health 2010, G. Csóka, pers. comm.). As testing of more than ten species of non-target arthropods may be impractical and often unnecessary (Sands 1997) and since it is belie-

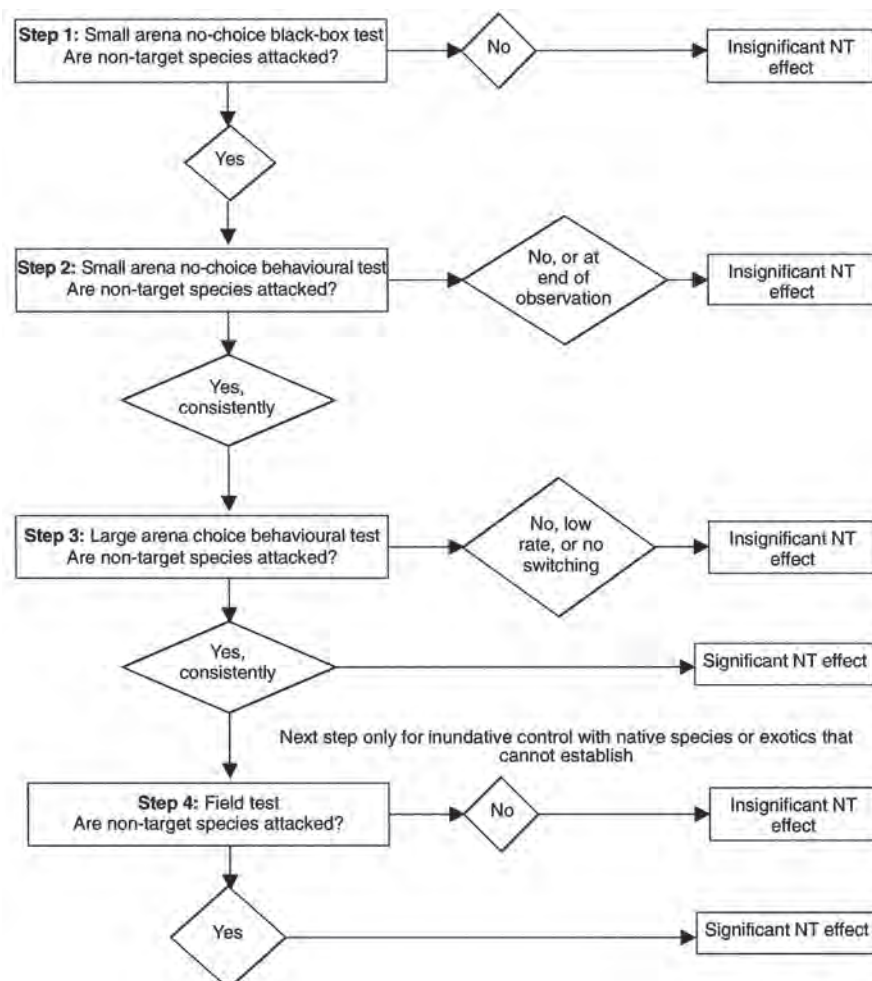


Figure 8. Flow chart describing host-range assessment (NT= non-target) (van Lenteren *et al.* 2006).

**Table 4 Species to be used in a host-range testing for *T. sinensis* (EFSA Panel on Plant health 2010) and host oak associations of western palaeartic oak cynipids**

Gallwasp species	Oak Section <i>Cerris</i>										Oak Section <i>Quercus</i>												
	Bra	Cas	Cer	Coc	Ile	Ith	Lib	Su	Tro	Can	Dal	Fag	Fra	Har	Inf	Lus	Mac	Pet	Pon	Pub	Pyr	Rob	
	Species	Gen	Sex	Sex	Sex	Sex	Sex	Sex	Sex	Sex	Sex	Sex	Sex	Sex	Sex	Sex	Sex	Sex	Sex	Sex	Sex	Sex	Sex
<i>Andricus curvator</i>			?							◆	◆	◆	◆	◆	◆		◆	◆		◆	◆	◆	◆
<i>Andricus cydoniae</i>			◆					?							◆								?
<i>Andricus grossulariae</i>			◆		◆		◆	◆															
<i>Andricus inflator</i>			?							◆			◆				◆			◆			◆
<i>Andricus lucidus</i>			◆					◆															
<i>Andricus multiplicatus</i>			◆				◆			◆													
<i>Biorhiza pallida</i>			?					?					◆		◆								◆
<i>Dryocosmus cerriphilus</i>			◆							◆			◆		◆								◆
<i>Neuroterus q.baccarum</i>			?							◆			◆		◆								◆

Records we consider doubtful are indicated by a question mark. Only confirmed hosts are given. The oak species name abbreviations are as follows: In the section *Cerris*, Bra=*Q. brantii*, Cas=*Q. castaneifolia*, Cer=*Q. cerris*, Coc=*Q. coccifera*, Ile=*Q. ilex*, Ith=*Q. ithaburensis*, Lib=*Q. libani*, Su=*Q. suber*, Tro=*Q. trojana*. In the section *Quercus sensu stricto*, Can=*Q. canariensis*, Dal=*Q. dalechampii*, Fag=*Q. faginea*, Fra=*Q. frainetto*, Har=*Q. hartwissiana*, Inf=*Q. infectoria*, Lus=*Q. lusitanica*, Mac=*Q. macranthera*, Pet=*Q. petraea*, Pon=*Q. pontica*, Pub=*Q. pubescens*, Pyr=*Q. pyrenaica*, Rob=*Q. robur*. Oak species in bold italic are the species found in Ticino.

ved that a carefully designed host-specificity test on a few species related to the target species should provide adequate and trustworthy information (Sands 1998) we recommend using the list provided here to perform host-range assessment for *T. sinensis* following the scheme developed by van Lenteren and colleagues (2006).

Chestnut and oak trees share common habitats. Oak presence was recorded in order to evaluate proximity with infested chestnut stands, and the possibility of contact between *T. sinensis* and oak galls in case of its release or natural immigration in Ticino. No pure oak forests were recorded in the studied areas. However, mixed stands, generally with *Robinia pseudoacacia* L., were recorded in Area A, D and E. In two areas (D and E) cynipid galls, most probably of *Andricus caputmedusae* Hartig were recorded on oak. Single oak trees were detected in several areas, being generally favoured by forestry management. The most frequent oak species is *Quercus robur* L., however, other *Quercus* species are quite common in Southern Ticino, including *Quercus cerris* L., *Q. petraea* Liebl. and *Q. pubescens* Willd.. The exotic *Quercus ilex* L. and *Q. rubra* L. are commonly planted in park areas and can be found naturalised in forests. Pure oak forests in Ticino are generally located at the same altitude and adjacent to chestnut forests, on southern slopes in dry locations and on limestone or ultra basic rocks (Figure 5). Table 4 describes the association among oak species present in Ticino and oak gall wasps to be used in host-range tests.

**Table 5. Parasitoids recruited by *D. kuriphilus* in Italy with origin and natural distribution (Modified after Aebi et al. 2007)**

Parasitoid species	Family	Origin	Distribution
<i>Sycophila iracemae</i>	Eurytomidae	No	M
<i>Sycophila variegata</i>	Eurytomidae	No	WP
<i>Sycophila biguttata</i>	Eurytomidae	No	WP
<i>Eurytoma pistacina</i>	Eurytomidae	No	WP
<i>Eurytoma bruniventris</i>	Eurytomidae	No	WP
<i>Eurytoma sp. New species</i>	Eurytomidae	No	?
<i>Mesopolobus mediterraneus</i>	Pteromalidae	No	M
<i>Mesopolobus sericeus</i>	Pteromalidae	No	Wp
<i>Mesopolobus tarsatus</i>	Pteromalidae	No	M
<i>Torymus sinensis</i>	Torymidae	I	China, Korea
<i>Torymus auratus</i>	Torymidae	No	WP
<i>Torymus flavipes</i>	Torymidae	No	WP
<i>Torymus scutellaris</i>	Torymidae	No	WP
<i>Megastigmus dorsalis</i>	Torymidae	No	WP
<i>Eupelmus urozonus</i>	Eupelmidae	No	WP
<i>Baryscapus pallidae</i>	Eupelmidae	No	WP
<i>Ormyrus pomaceus</i>	Ormyridae	No	WP

No, native from oak cynipids; I, introduced WP, western palaeartic; M, Mediterranean Europe

Table 6. Guidelines and procedure for host-range testing of the chestnut gall wasp parasitoid <i>T. sinensis</i> (Modified from Kuhlmann et al. 2006)						
Step	Question to be answered	Positive control	Negative control	Parameters to be measured	Methods for analysis	Interpretation
<b>Step 1: Small arena no-choice behavioral test.</b>	Does <i>T. sinensis</i> attack oak galls? If yes, to what extent?	<i>D. kuriphilus</i> galls with <i>T. sinensis</i> to check <i>T. sinensis</i> ' ability to oviposit	<i>D. kuriphilus</i> and oak galls without <i>T. sinensis</i> , to check survival of oak gall wasp under test conditions.	Number of galls parasitized and not parasitized (stinging, oviposition) Number of galls parasitized and not parasitized (dissection, emergence of <i>T. sinensis</i> ). Host suitability for <i>T. sinensis</i> (host-location, antennating, drilling (oviposition or host-feeding) , rearing. Encounter and attack rate of oak galls over time to determine possible increase in acceptance due to increasing oviposition pressure. Latency time to first attack	To compare the proportion of <i>D. kuriphilus</i> and oak galls attacked generalized linear model should be used.	If no oak galls are attacked and the positive control is validated ( <i>D. kuriphilus</i> galls were attacked at a rate approaching that in the field), no direct effects on the tested oak galls are expected in the field.  If <i>D. kuriphilus</i> and oak galls are attacked, but the oak galls attack rate is significantly lower than the <i>D. kuriphilus</i> gall attack rate, risk of oak gall attack may be low but further testing should be considered (step 2)  If oak galls are attacked only at the end of the observation period, then the risk of direct effect on oak galls is low.  If oak galls are consistently attacked with a latency time (time between exposure to oviposition) similar to <i>D. kuriphilus</i> galls, and attack of <i>D. kuriphilus</i> and oak galls do not differ significantly, further testing should be considered (step 2).
<b>Step 2: Large arena choice behavioral test.</b>	Does <i>T. sinensis</i> attack oak galls when <i>D. kuriphilus</i> and oak galls are present in a semi-natural situation?	<i>D. kuriphilus</i> galls with <i>T. sinensis</i> to check <i>T. sinensis</i> ' ability to oviposit Oak galls with <i>T. sinensis</i> ( identical number of galls at the start of each experiment, in each control)	<i>D. kuriphilus</i> and oak galls without <i>T. sinensis</i> , to check survival of <i>D. kuriphilus</i> under test conditions.	As in step 1.	As in step 1.	If oak galls are often attacked (with similar latency time and attack rate than <i>D. kuriphilus</i> galls), high oak gall attack can be concluded.  If latency times of attack of oak gall are much higher and attack rates are much lower than in the <i>D. kuriphilus</i> control, <i>T. sinensis</i> shows a strong preference for <i>D. kuriphilus</i> , but may still attack oak galls in situations where <i>D. kuriphilus</i> is not present.  If latency times of attack of oak gall are much higher than in the <i>D. kuriphilus</i> control and the attack rates are much lower in the choice tests and oak gall control, the risk of direct effects on oak galls under field conditions is small.

The community centered on oak and chestnut galls is species rich. A whole suit of parasitoids (Hymenoptera: Chalcidoidea) and inquilines (Hymenoptera: Cynipoidea) attack oak galls (Stone *et al.* 2002; see appendix 3). Although it was believed that gall inducers using different host-plants had specific guilds of natural enemies, there is ample evidence that parasitoid species are able to attack galls on different host plants (Aebi *et al.* 2006, 2007) when host-plants coexist. The oak gall wasp parasitoids that were recruited by the chestnut galls are listed in table 5. It is striking that 16 species from 4 families were able to use another gallwasp species on another host-plant as a host. More worrying is the fact that several *Torymus* species (*T. auratus*, *T. flavipes* and *T. scutellaris*) showed no host-plant fidelity and searched hosts on another plant. These observations suggest that *T. sinensis* also has the potential to search for suitable hosts on another host plant, namely galls induced on oaks. The aim of host-specificity testing for *T. sinensis* is to assess its the potential host-range, in other words to demonstrate whether *T. sinensis* can attack oak gall wasps on another host-plant. A generally accepted testing scheme is presented in figure 8. However experiments had to be adapted to this particular biological system. Guidelines and procedure for host-range testing for *T. sinensis* are summarized in table 7. The aim of this experimental procedure is to evaluate oak galls attack by *T. sinensis* in choice and no-choice experiments. The use of adequate positive and negative controls is crucial for a sound interpretation of the results. Advices on the interpretation of the results and evaluation of non-target environmental hazards are provided in table 6. However, before performing these tests it might be useful to contact the competent authority (FOEN) in order to verify whether these procedures are consistent and sufficient according to Swiss law.

Table 7. Guidelines and procedure for the evaluation of hybridization risk between *T. sinensis* and native parasitoids. (Modified from Hopper *et al.* 2006)

Step	Question to be answered	Positive control	Parameters to be measured	Methods for analysis	Interpretation
<b>Step 1: Mate recognition in the laboratory</b>	Do <i>T. sinensis</i> and congeneric species recognize themselves as mates in laboratory conditions?	Within species crosses to check if these species do recognize themselves as mate in laboratory conditions	Number of couples courting. Number of couples mating. Crosses involving males and females from both species should be used.	To compare the proportion of couples mating and couples not mating, simple statistical tests should be used.	If <i>T. sinensis</i> and native species court and couple only with conspecifics in the laboratory, this is likely to hold in field conditions, and no risk of hybridizations are foreseen.  If <i>T. sinensis</i> and native species court and couple with one another in the laboratory, this may or may not mean that they will do so in the field. Further testing should be considered (step 2)
<b>Step 2: Copulation and sperm transfer in the laboratory</b>	Does mating between <i>T. sinensis</i> and congeneric species lead to sperm transfer in laboratory conditions?	Within species crosses to check if sperm is transferred to the females in laboratory conditions	Number of females inseminated. Crosses involving males and females from both species should be used.	Female dissection and sperm molecular staining.  To compare the proportion of females being inseminated and females not being inseminated simple statistical tests should be used.	If mating between <i>T. sinensis</i> and native species in the laboratory does not lead to sperm transfer, no risk of hybridizations are foreseen.  If mating between <i>T. sinensis</i> and native species in the laboratory leads to sperm transfer, further testing should be considered (step 3)
<b>Step 3: Production of viable hybrids in the field</b>	Does mating between <i>T. sinensis</i> and congeneric species lead to the production of viable offspring in the field?	Not applicable	Number of hybrid detected.	Molecular analysis on wild caught <i>Torymus</i> specimens. Insertions/deletions in nuclear ribosomal genes like ITS1 and ITS2.	If hybrid never or rarely occur in the field, little risks associated with hybridization are foreseen.  If hybrid frequently occur in the field, introgressions may be possible and high risks associated with hybridization are foreseen.



## 9. Evaluating risk of hybridization between the introduced *Torymus sinensis* and native oak gall wasp parasitoids

In theory, insect species introduced for biological control may interbreed with native species. The only reported case to date involves the chestnut gallwasp parasitoid *T. sinensis* and a Japanese native *Torymus* species; *T. beneficus*. Hybridization was suspected (Shiga, 1999) and in 1992, Moriya and colleagues successfully crossed *T. sinensis* and *T. beneficus* in the laboratory to produce fertile hybrid females. Hybrids were also detected in the field (Moriya *et al.*, 1992, 2003; Yara *et al.*, 2000) and molecular markers proved their hybrid origin (Izawa *et al.*, 1992, 1995, 1996; Toda *et al.*, 2000; Yara, 2004, 2006; Yara *et al.*, 2000). Here we follow the recommendations of Hopper and colleagues (2006) to establish an experimental procedure for the evaluation of hybridization risks between *T. sinensis* and native parasitoids. Sixteen native oak gall wasp parasitoids from 4 families were consistently reared from the chestnut gall wasp, *D. kuriphilus* (Table 5). Among them, three *Torymus* species (*T. auratus*, *T. flavipes* and *T. scutellaris*) have a clear potential to hybridize with *T. sinensis* as the answer to the first three question of the pre-introduction test flowchart (Figure 9) is yes. Indeed, closely related species

do overlap geographically (they may even parasitize identical galls on single chestnut trees) and probably overlap in their seasonality.

We propose to combine laboratory and field experiments to answer the following questions about potential hybridization between *T. sinensis* and congeneric species, namely mate recognition, copulation and sperm transfer and the viability of hybrids (Table 8).

The main difficulty relies on the fact that laboratory observations may not be transposable to field conditions and that univoltine species developing on galls are impossible to rear in laboratory conditions. Mate recognition, mating and potential sperm transfer have to be evaluated in laboratory experiments on individuals reared from field collected galls. If these steps are validated, one need to perform molecular analyses on individuals reared from field collected galls, to infer the hybridization frequency in the environment. Because of the impossibility to rear these species, introgression events and subsequent potential effect on host-range, climatic tolerance, mating behavior and other traits of interest would be extremely difficult to investigate. Advices on the interpretation of the results and evaluation of non-target environmental hazards are provided in table 8. However, before performing these tests it might be useful to contact the competent authority (FOEN) in order to verify whether these procedures are consistent and sufficient according to Swiss law.

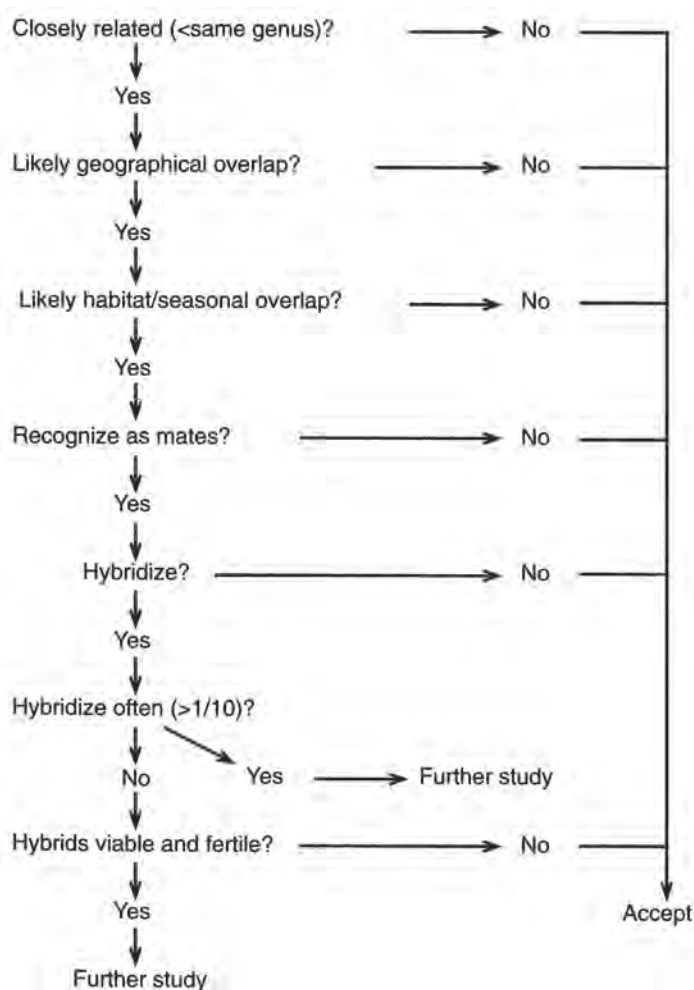


Figure 9. pre-introduction tests to predict interbreeding between species introduced for biological control and native species (Hopper *et al.* 2006).

## 10. Further taxonomic difficulties with *T. sinensis*

The identification of closely related species can be difficult among hymenopteran parasitoids (Aebi, unpublished data; Davis *et al.* 1987; Hoy *et al.* 2000). As a consequence, a taxon described as a species may actually consist of a set of sibling species or ecotypes which could differ in traits such as host-specificity (Davis *et al.* 1987). Work in Korea and Japan has shown that *T. sinensis* is part of a taxonomically complex set of closely related species that are biologically diverse, but difficult to distinguish morphologically (Murakami, 1988; Yara *et al.* 2000; Yara 2004). Native Korean parasitoids identified morphologically as *T. sinensis* can be divided into two ecotypes on the basis of their adult emergence periods (Murakami *et al.* 1995). The two ecotypes are thought to be derived from native oak cynipid hosts with differing phenology (Murakami *et al.* 1995).

Female morphology has been used in the past to distinguish *T. sinensis* and *T. beneficus*. The ratio of the ovipositor sheath length to the thorax length (O/T ratio; Ôtake, 1987) in combination with the adult emergence time was used to identify the two species. *T. sinensis* has a larger O/T ratio than *T. beneficus* (Otake, 1987). *T. sinensis* females emerge later than *T. beneficus* females (5<sup>th</sup> to 23<sup>rd</sup> of April and 10<sup>th</sup> of March to 8<sup>th</sup> of April, respectively, Murakami, 1981). However, analyses by Yara (2004) using Cytochrome oxidase I sequence data (a marker widely used in molecular systematics; Caterino *et al.* 2000) have shown that the O/T ratio is an unreliable character in discrimination of *T. sinensis* and *T. beneficus*. An additional twist in the tale is the demonstration of three distinct ecotypes in Japanese *T. beneficus*, whose adults differ in their emergence phenology between an early season emerging strain (TbE, second half of March to first half of April), a late-season emerging strain (TbL, late April to early May) and an autumn strain (TbA, October to November; Otake, 1987; Murakami, 1988; Yara, 2004). All three strains attack *D. kuriphilus* in Japan, but are thought to have originated from different oak cynipid hosts. We thus strongly encourage to use a molecular based approach to identify the strain of *T. sinensis* used for any behavioural test evaluating potential non-target environmental effects.

## 11. Potential for biological control using native parasitoids

Native parasitoids could potentially be exploited in either augmentative biological control (rearing and subsequent release of native parasitoids) or conservation biological control (an approach used in closed spaces such as breeding cages or granaries) to enhance native parasitoid populations. Sixteen parasitoid species have so far recruited naturally to *D. kuriphilus* in Italy (Table 8). Early recording of parasitoid communities in Italy captured the rapid recruitment process. Community richness rose from 2 species in 2002, to 7 in 2003, 10 in 2004 and 15 in 2005. Despite the increase in community richness, gall attack rates (estimated per inhabitant) have remained very low: from 1.6% in 2003 (n= 1900 galls), to 0.8% in 2004 (n= 2500) and 0.5% in 2005 (n= 6713). Of the parasitoids attacking *D. kuriphilus* in Italy, the most significant and consistent is *Eupelmus urozonus*. The rather low attack rate may well be because *D. kuriphilus* galls develop midway between the spring and summer/autumn generations characteristic of oak cynipids, resulting in a mismatch between emergence of native parasitoid adults and chestnut galls in an appropriate developmental stage for attack (Aebi et al., 2006, 2007). The rapid recruitment of oak cynipid parasitoids to *D. kuriphilus* suggests that despite current low attack rates there may be some value in an augmentative or conservation biological control programme using native species. Here again, accurate taxonomic assessment of the component species is essential for the reasons described

above. Recent phylogeographic work has shown the existence of cryptic lineages in several taxa previously regarded as single species, including *Eurytoma brunniventris* (Eurytomidae), *Megastigmus dorsalis* (Torymidae) and *Euplemus urozonus* (Euplemidae). The biology of these cryptic lineages is poorly understood. Current knowledge suggest that they are not host plant specific, but attack

**Table 8. Parasitoids attacking *D. kuriphilus* in Italy with date of first record (date), geographic distribution in the Western Palearctic and the proportion of parasitism made up by each species in Italian rearings in 2003, 2004 and 2005.**

Parasitoid species	Family	Date	Dist	2003	2004	2005
<i>Sycophila iracemae</i>	Eurytomidae	2004	M		4	
<i>Sycophila variegata</i>	Eurytomidae	2003	WP	2		
<i>Sycophila biguttata</i>	Eurytomidae	2005	WP			3
<i>Eurytoma pistacina</i>	Eurytomidae	2004	M		1	8
<b><i>Eurytoma brunniventris</i></b>	Eurytomidae	2002	WP		2	
<i>Eurytoma sp. New species</i>	Eurytomidae	?	?	*	*	*
<i>Mesopolobus mediterraneus</i>	Pteromalidae	2004	M		1	1
<i>Mesopolobus sericeus</i>	Pteromalidae	2003	WP	5		
<i>Mesopolobus tarsatus</i>	Pteromalidae	2005	M			1
<i>Torymus auratus</i>	Torymidae	2005	WP			1
<i>Torymus flavipes</i>	Torymidae	2003	WP	2	5	1
<i>Torymus scutellaris</i>	Torymidae	2005	WP			1
<b><i>Megastigmus dorsalis</i></b>	Torymidae	2002	WP	14		7
<b><i>Eupelmus urozonus</i></b>	Eupelmidae	2002	WP	77	87	77
<i>Baryscapus pallidae</i>	Eupelmidae	2005	WP	*	*	*
<i>Ormyrus pomaceus</i>	Ormyridae	2002	WP	*	*	*

Dist: WP= western palearctic; M= Mediterranean Europe

Abundance estimates were unavailable (\*) for *B. pallidae*, *O. pomaceus* and *Eurytoma sp. New species*. Species with known cryptic lineages are in **bold** (Modified after Aebi et al. 2006)

overlapping sets of hosts on shared host plants. The extent to which they interbreed and are biologically different (as in the *T. sinensis* complex) is unclear. Further molecular and ecological studies are crucially needed to further evaluate the potential of native parasitoid for the control of *D. kuriphilus*.



## 12. Preliminary conclusions

The invasion of *D. kuriphilus* in Switzerland is at its initial stage. On the southern side of the Alps, it will probably spread quickly and colonise most chestnut forests within a few years. Dispersion to chestnut populations located North of the Alps is only a matter of time, unless control methods lower the pest's density and slow down its spread. In Switzerland, cultural value of chestnut is very high and undergoing an important revival in Ticino, around Lucerne and in the Chablais with traditional chestnut groves and social activities around chestnuts. In case of heavy infestation and great visibility of *D. kuriphilus*, public pressure on the authorities to reduce its population may be important. Both economic and ecological damage may be significant, even if difficult to evaluate precisely. In the light of this threat, it seems appropriate to explore the possibility of the introduction of *T. sinensis* for biological control. *Torymus sinensis* was shown to cause post-release, unwanted non-target effect on the environment in Japan. There is an urgent need to evaluate the environmental risks associated with a potential release of *T. sinensis* in Ticino. In particular, attention should be given to the beneficial insect's host-range and to risks of hybridisation with European *Torymus* species centred on oak gall wasps. For this purpose, experimental procedures were developed to fulfil this gap. Overall, data on environmental impact of *T. sinensis* would complete the available information on the presence of *D. kuriphilus* in Switzerland, its infestation rate, its potential ecological and economical impact and on the effect of the release of *T. sinensis* in Italy. Once available, this information would place us for the first time in the position of performing a complete risk assessment which allows for a true cost and benefit analysis for the use of *T. sinensis* against the invasive chestnut gall wasp, threatening chestnuts in several European countries.

## 13. Next steps and possible roadmap

To complete a risk assessment for the use of *T. sinensis* against the invasive chestnut gall wasp (which would allow a true benefit/cost/risk assessment) it is crucial to gather data on the following aspects:

- Measurements of future *D. kuriphilus* infestation in reference surfaces in Ticino.
- Measurements of damage reductions achieved with the release of *T. sinensis* in Italy.
- Monitoring of the natural arrival of *T. sinensis* in Switzerland.
- Evaluation of *T. sinensis*' host-range.
- Evaluation of hybridization risks among *T. sinensis* and native *Torymus* species.
- Ecological studies on *T. sinensis*' adaptation to the European *C. sativa*.
- Ecological and molecular studies on potential use of native parasitoids against *T. sinensis*.
- Monitoring of the presence of *T. sinensis* on potential non-target hosts (oak galls) and molecular identification

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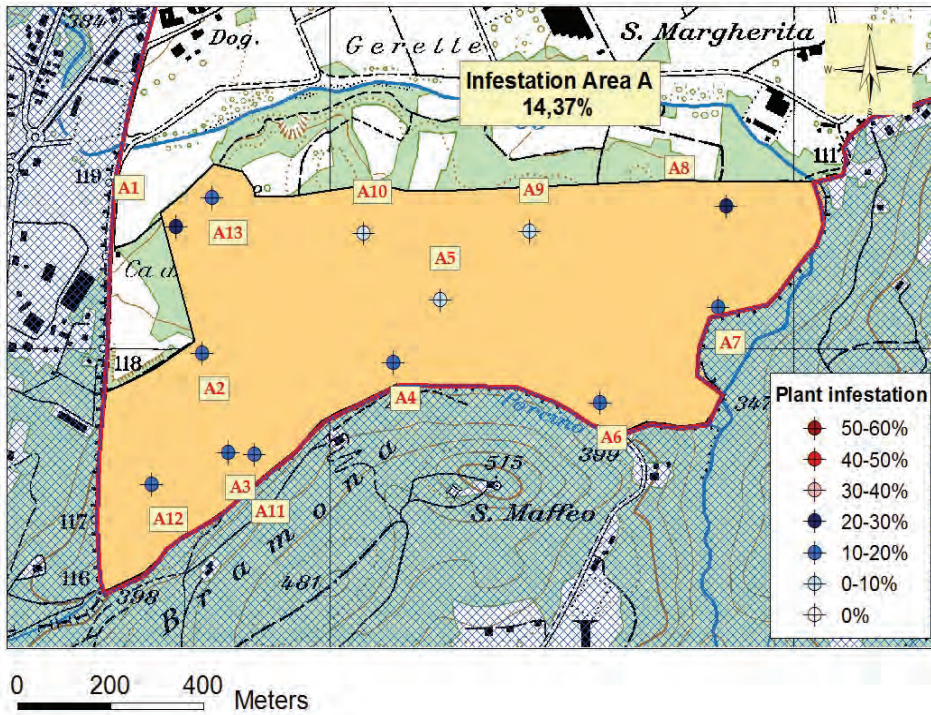
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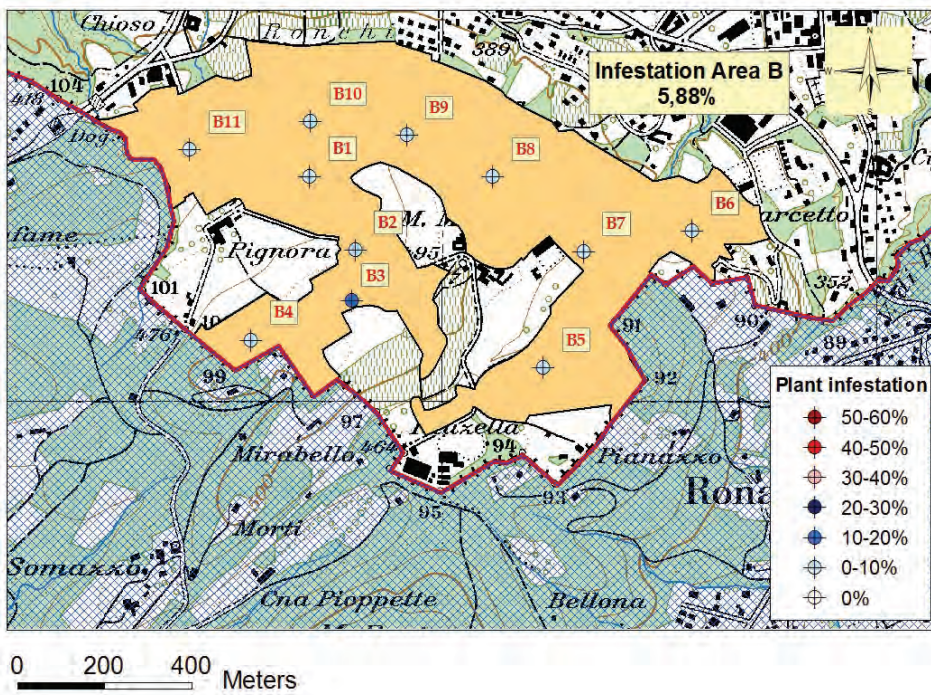


# 16. Appendices

## Appendix 1. Degree of infestation of single trees in each area



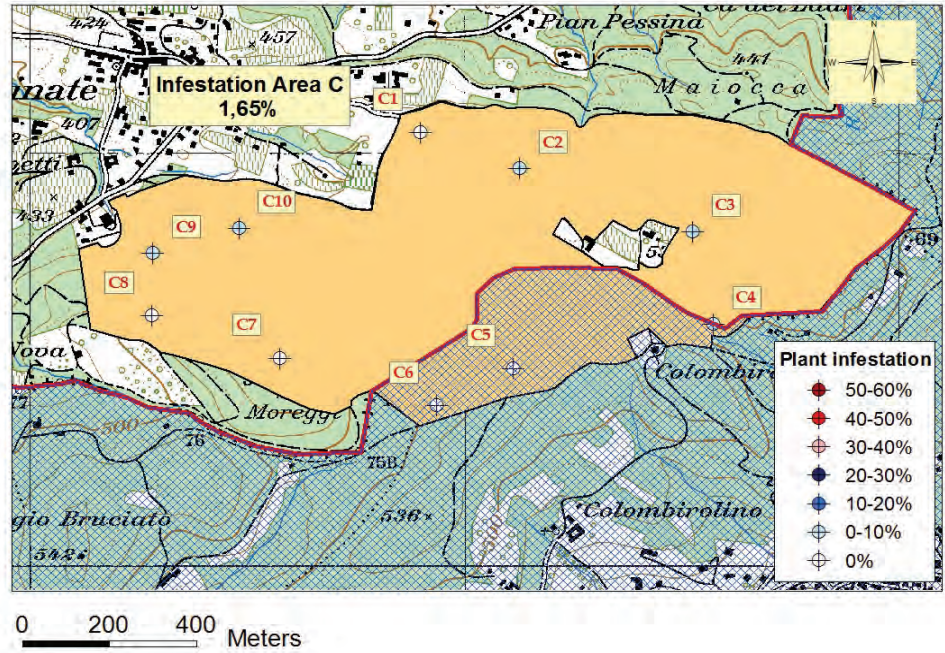
Area A: Gaggiolo



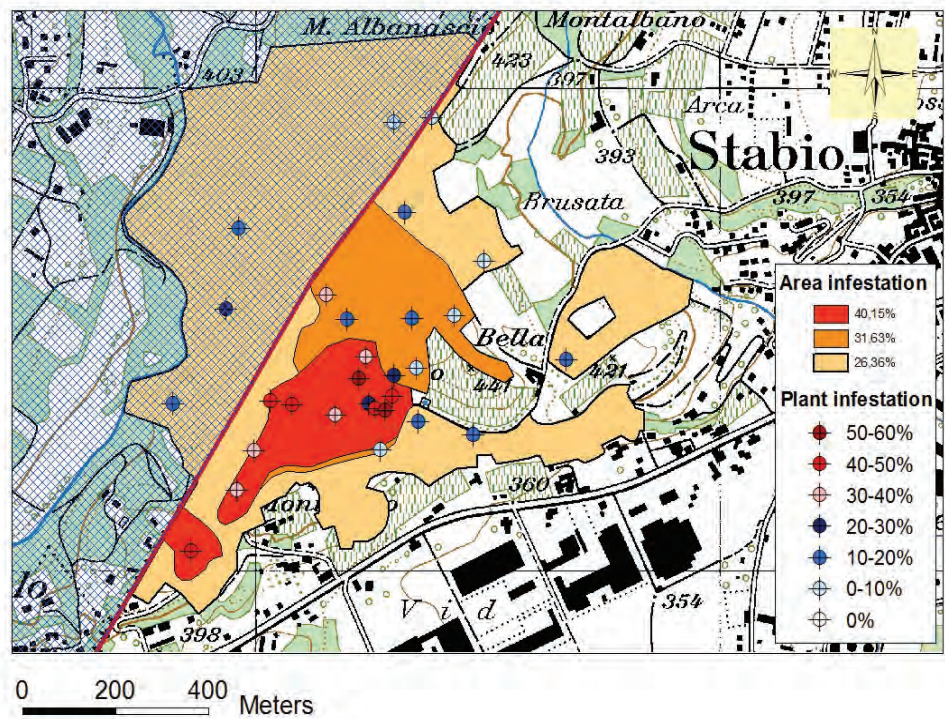
Area B: Casate

Italian territory: crosshatched blue surface



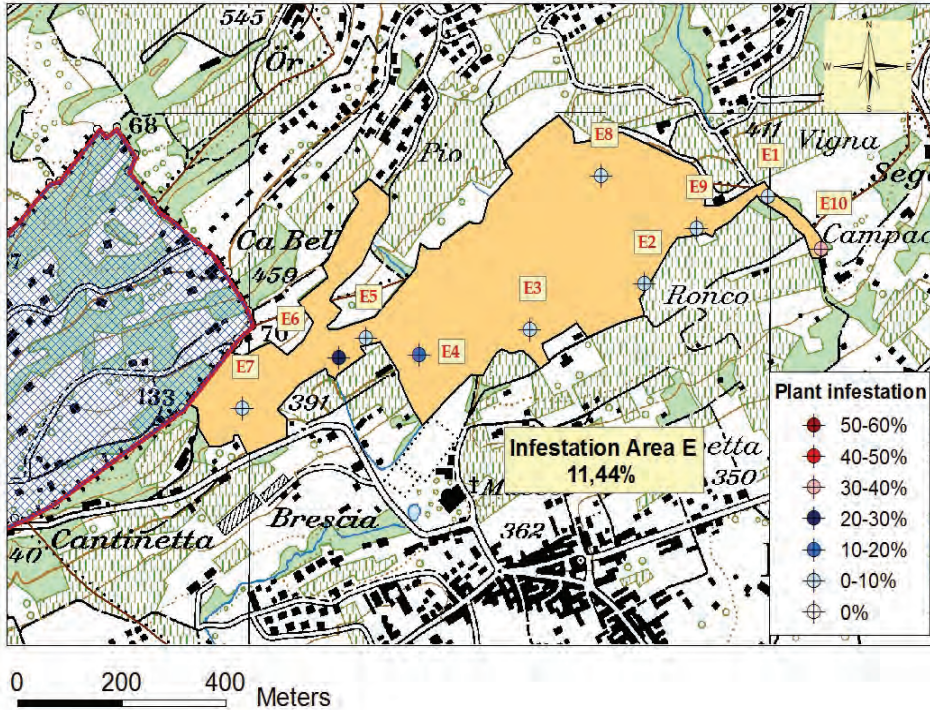


Area C: Pedrinate

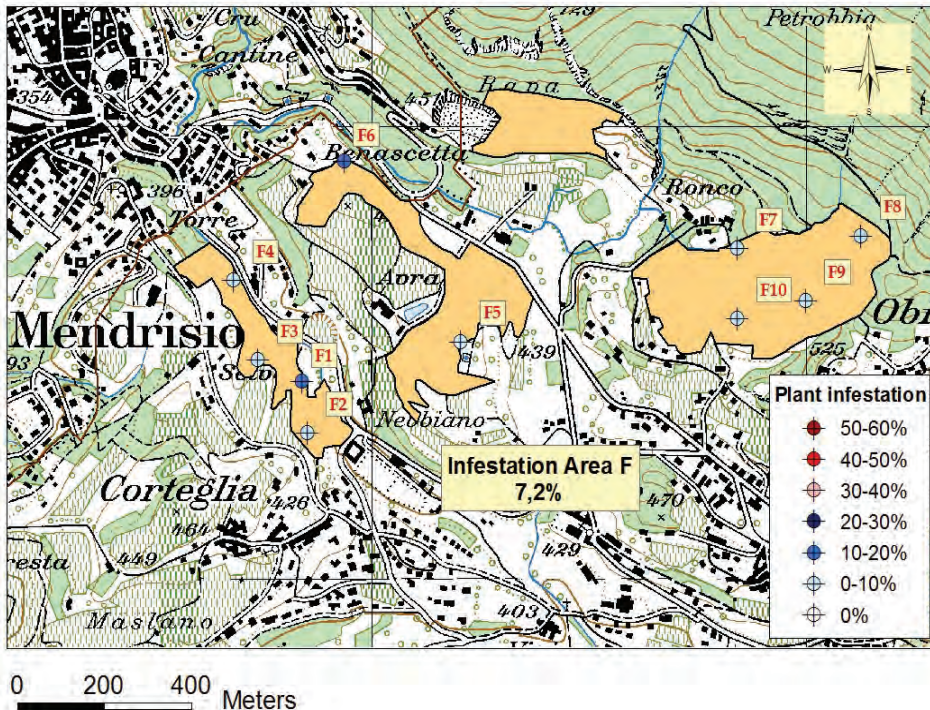


Area D: Stabio



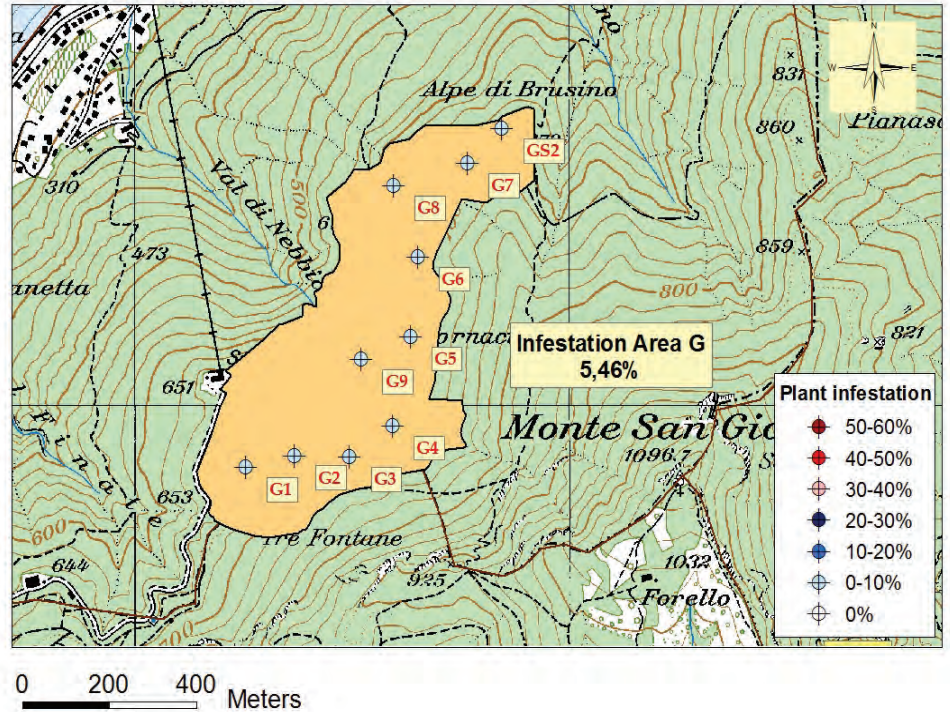


Area E: Rancate

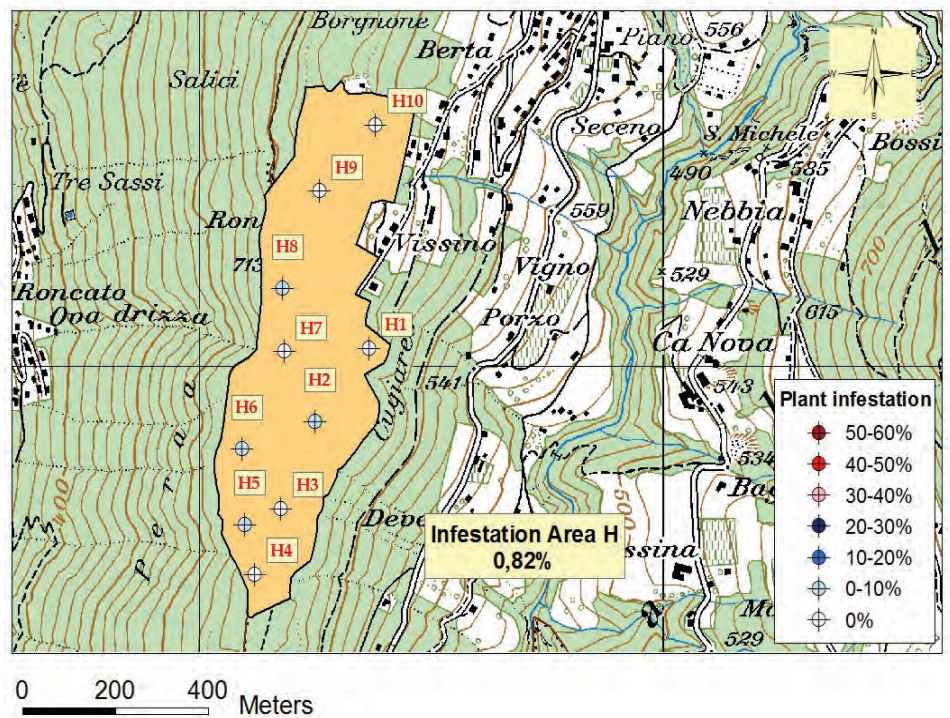


Area F: Mendrisio





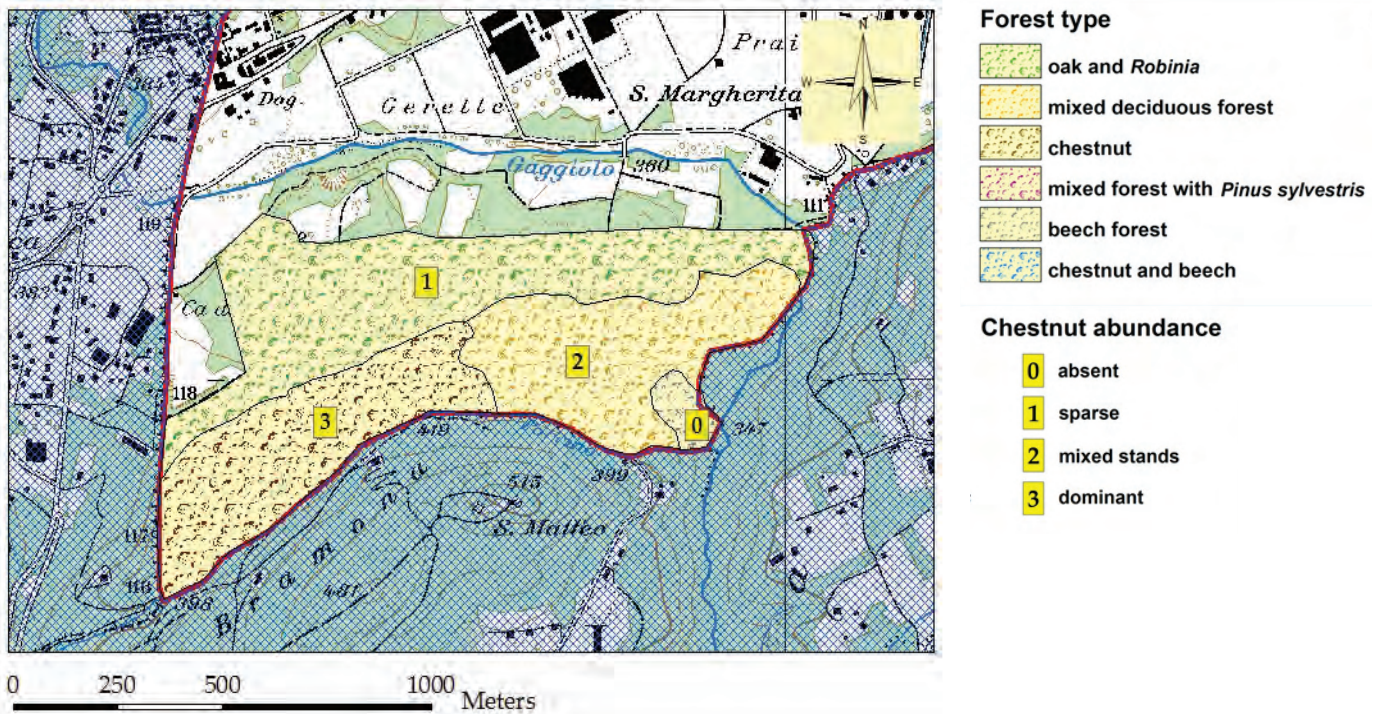
Area G: Serpiano



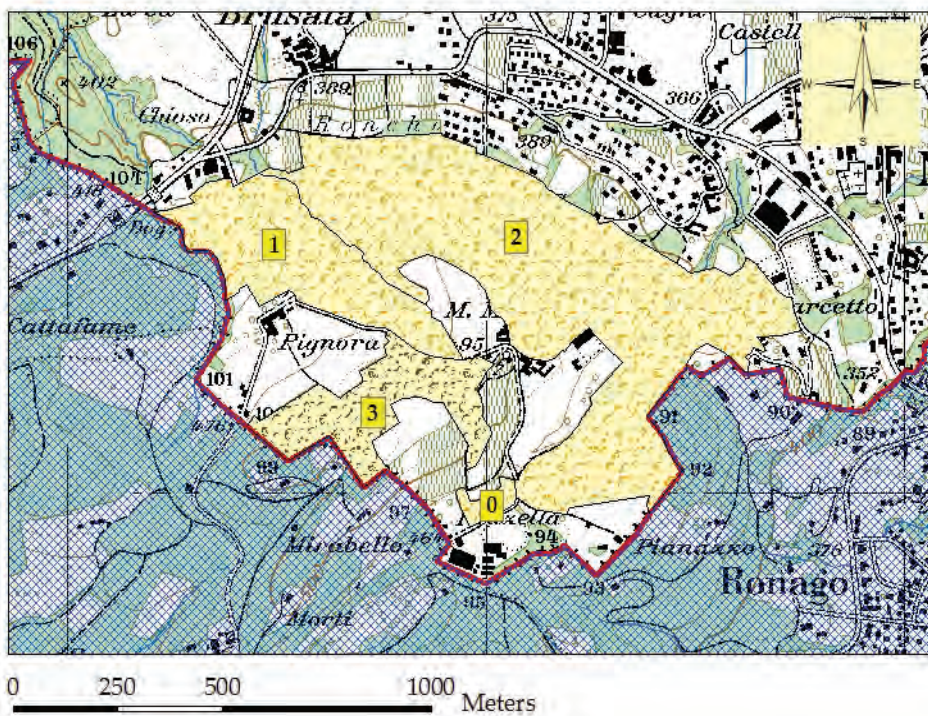
Area H: Arogno



Appendix 2. Forest type and relative chestnut density in each area



Area A: Gaggiolo









Area B: Casate

Italian territory: crosshatched blue surface

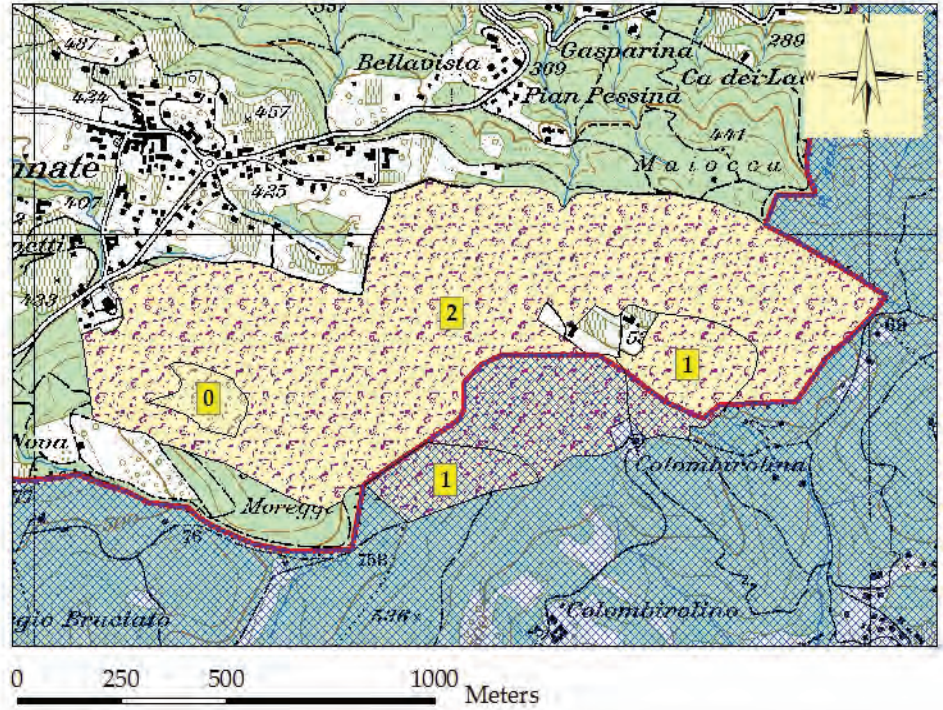


**Forest type**

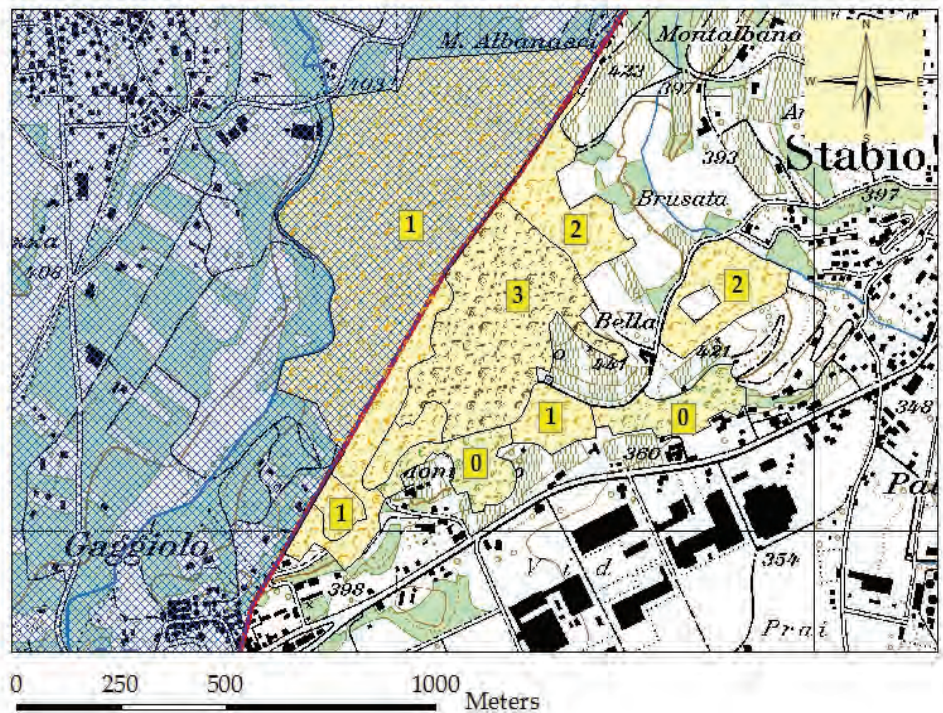
-  oak and *Robinia*
-  mixed deciduous forest
-  chestnut
-  mixed forest with *Pinus sylvestris*
-  beech forest
-  chestnut and beech

**Chestnut abundance**

-  0 absent
-  1 sparse
-  2 mixed stands
-  3 dominant

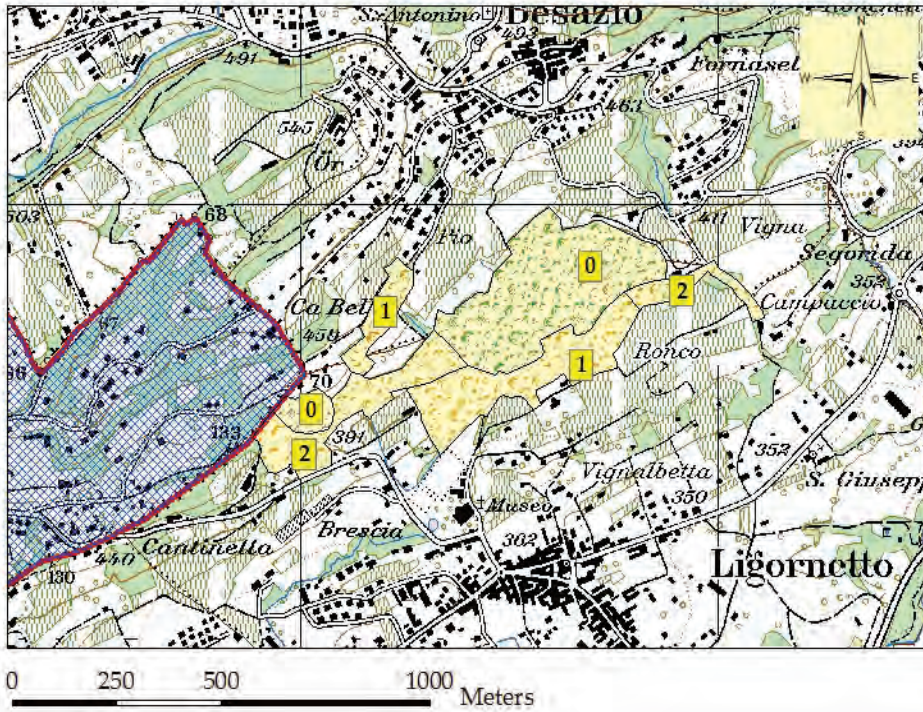


Area C: Pedrinate

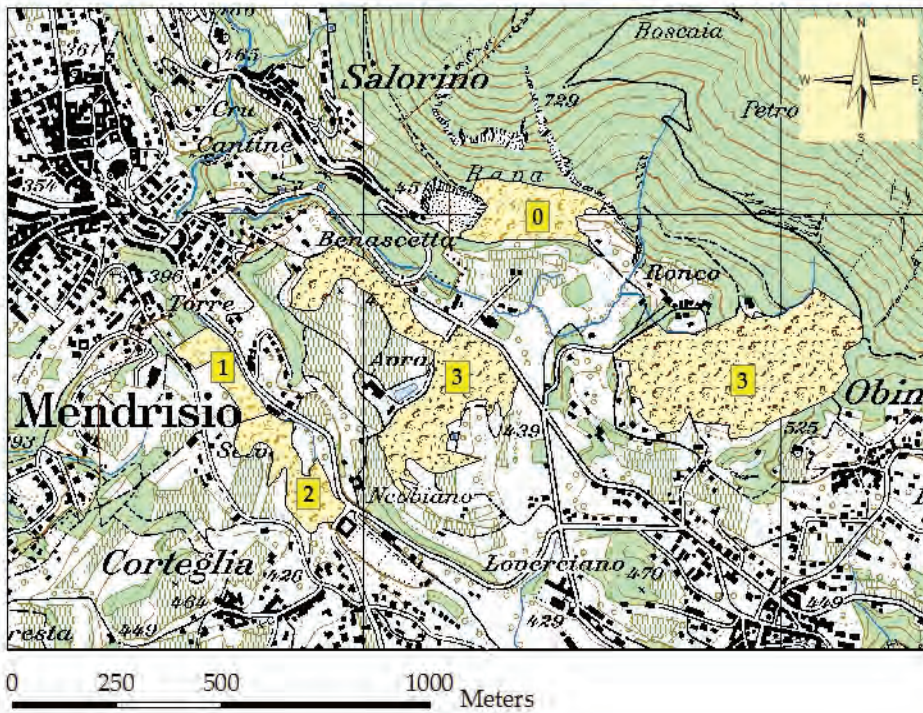


Area D: Stabio











Area E: Rancate



Area F: Mendrisio

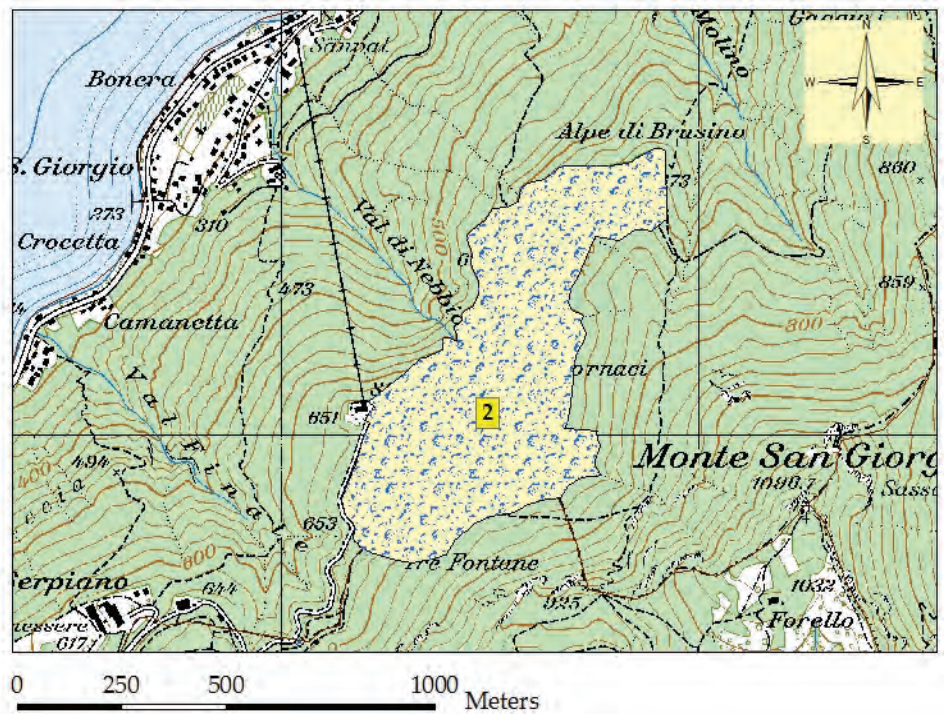


**Forest type**

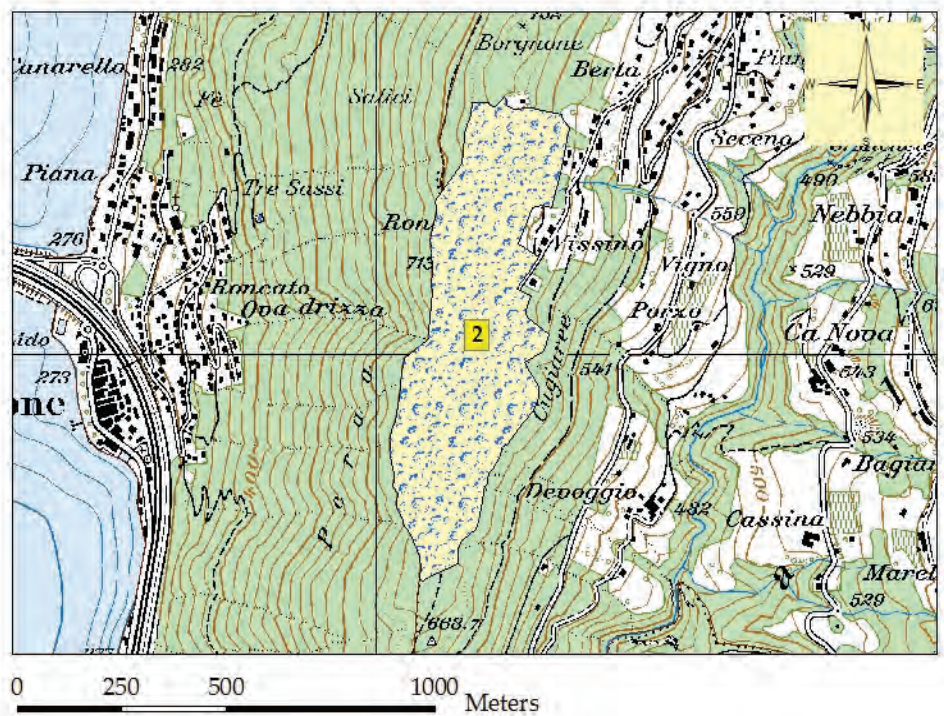
-  oak and *Robinia*
-  mixed deciduous forest
-  chestnut
-  mixed forest with *Pinus sylvestris*
-  beech forest
-  chestnut and beech

**Chestnut abundance**

-  0 absent
-  1 sparse
-  2 mixed stands
-  3 dominant



Area G: Serpiano

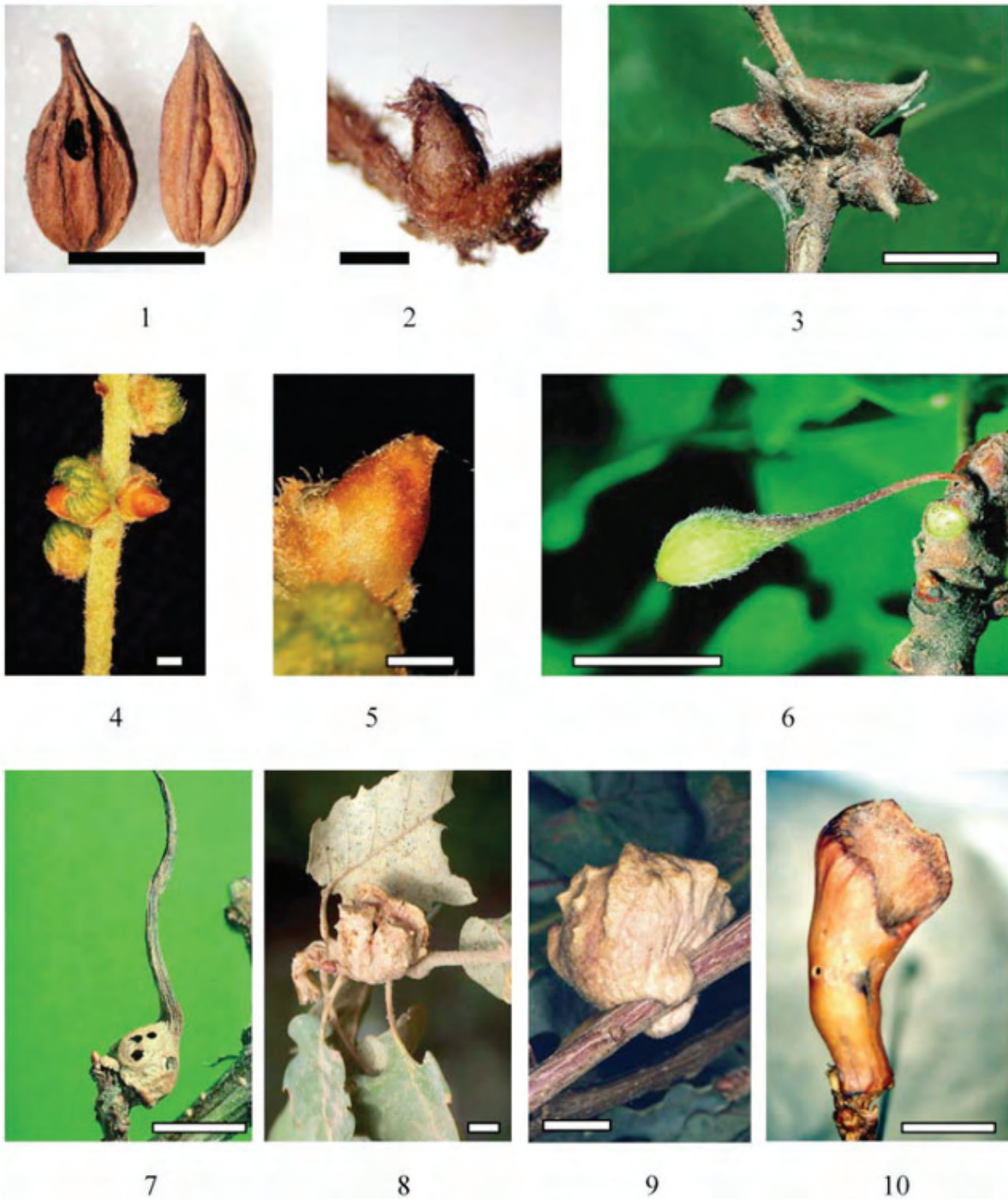


Area H: Arogno

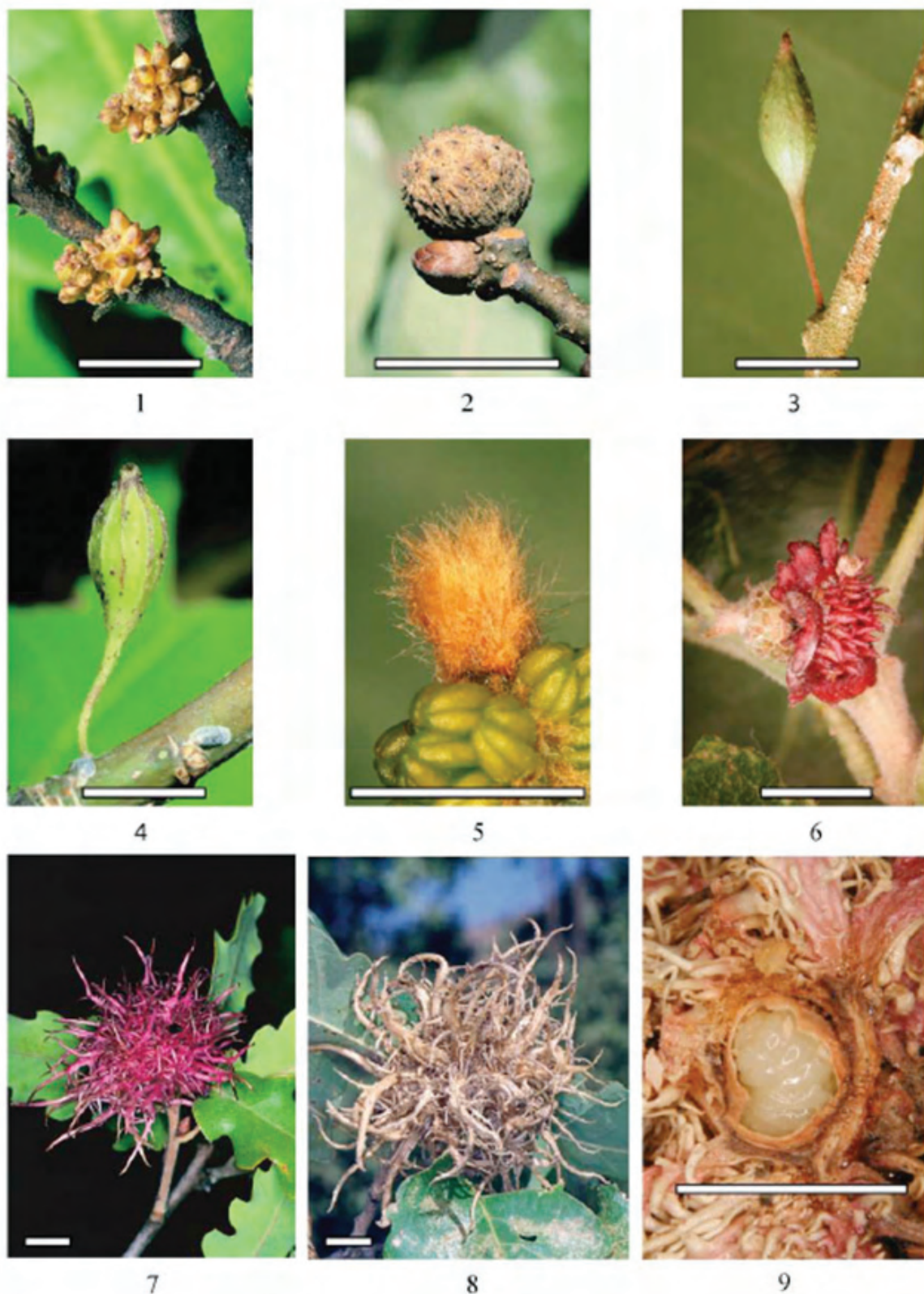


## Appendix 3. Oak gall diversity

(Courtesy of Dr. Graham Stone)



**Plate 1.** 1. *Andricus alniensis* asexual galls (scale bar 1 cm) ©JP. 2. *Andricus alniensis* sexual gall (scale bar 1mm) ©JP. 3. Cluster of *Andricus amblycerus* asexual galls (scale bar 5mm) ©GyCs. 4–5. *Andricus amenti* sexual galls (scale bar 1mm) ©GyCs. 6. *Andricus amenti* asexual gall (scale bar 1 cm) ©GyCs 7. *Andricus aries* asexual gall (scale bar 1 cm) ©GyCs. 8–9. *Andricus askewi* asexual gall (scale bars 1 cm) ©GS 10. *Andricus bulgaricus* asexual gall (scale bar 1 cm) ©GS.

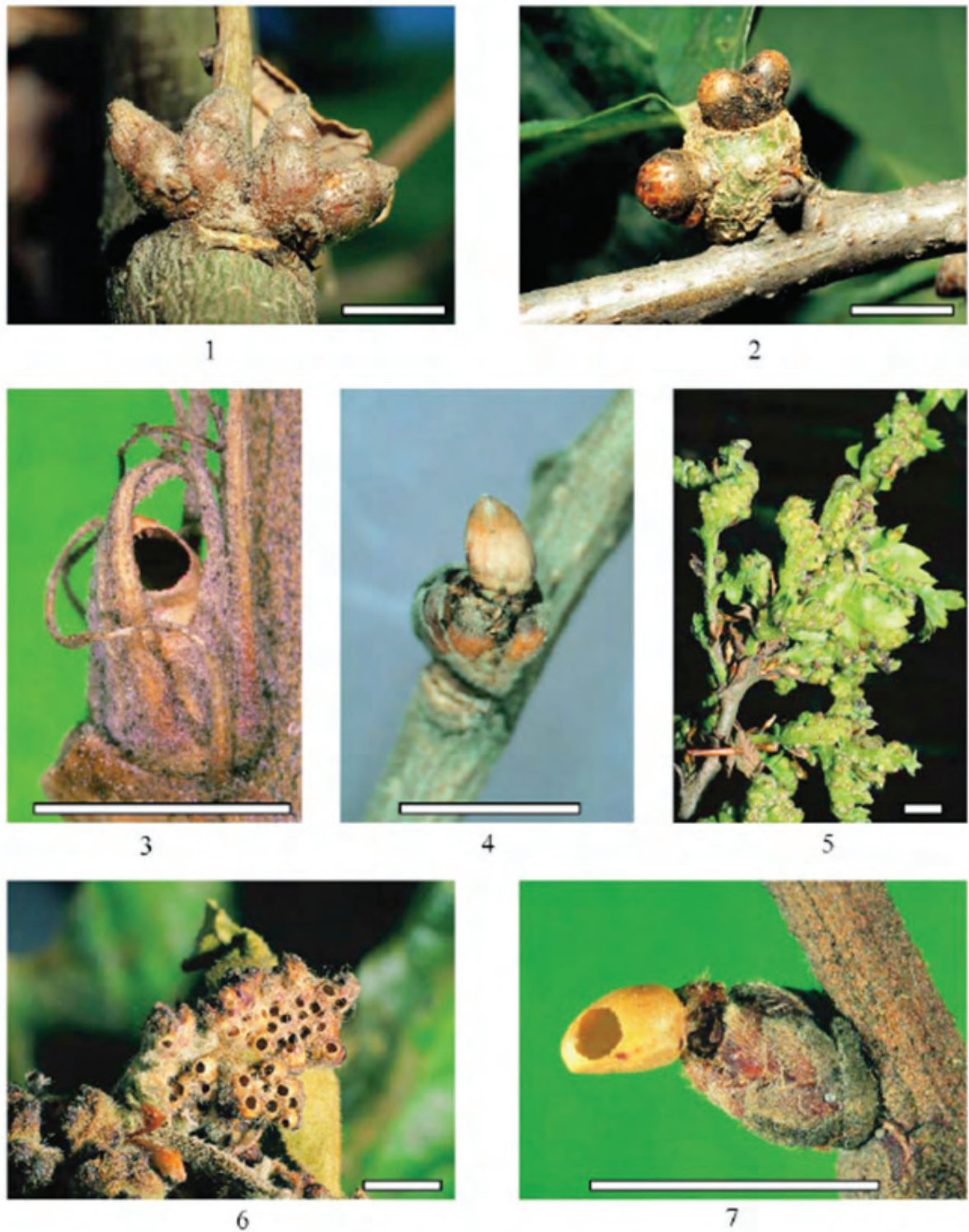


**Plate 2.** 1. *Andricus burgundus* sexual galls (scale bar 1 cm) ©GyCs. 2. *Andricus caliciformis* asexual gall (scale bar 1 cm) ©GyCs. 3–4. *Andricus callidoma* asexual gall (scale bars 1 cm) ©JLNA, GyCs. 5. *Andricus callidoma* sexual gall (scale bar 1 cm) © GyCs. 6. *Andricus caput-medusae* asexual gall, early in development (scale bar 1 cm) ©GyCs. 7–8. Fully developed *Andricus caput-medusae* asexual galls, mature in 8 (Scale bars 1 cm) ©GS. 8. *Andricus caput-medusae* larva in an opened asexual gall (scale bar 1 cm) ©GyCs.



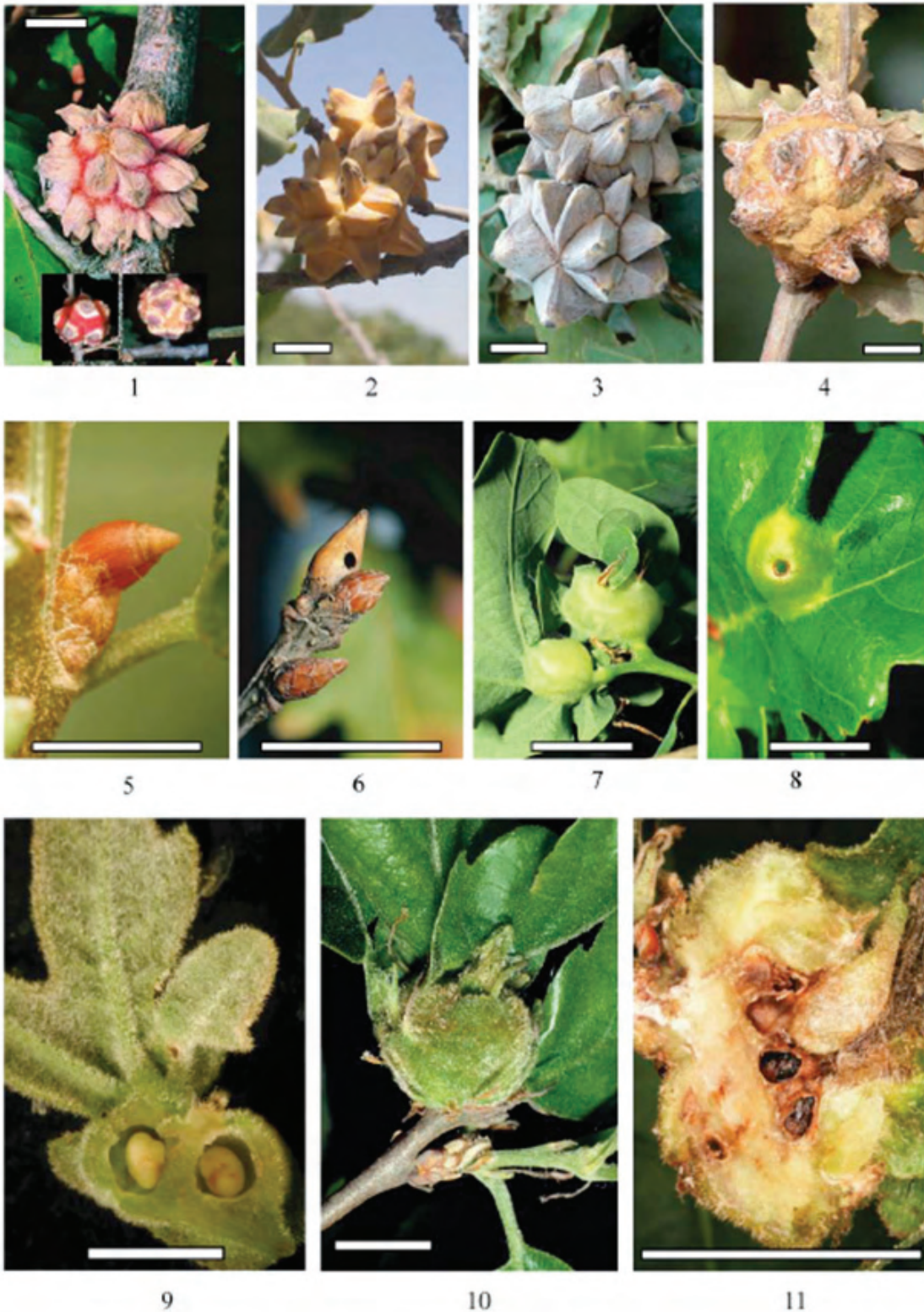


**Plate 3.** 1–4. *Andricus cecconii* sexual galls (scale bars 1 cm), young galls in 1,2, mature/emerged galls in 3,4 ©MT, MT,GyCs, MT. 5. *Andricus conglomeratus* asexual galls (scale bar 1 cm) ©GyCs. 6. *Andricus conificus* asexual gall when young (scale bar 1 cm) ©GyCs. 7. *Andricus conificus* asexual gall when mature (scale bar 1 cm) ©GyCs. 8–9. *Andricus coriarius* asexual gall (scale bar 1 cm) ©GyCs, GS. 10. *Andricus coronatus* asexual gall (scale bar 1 cm) ©GyCs.



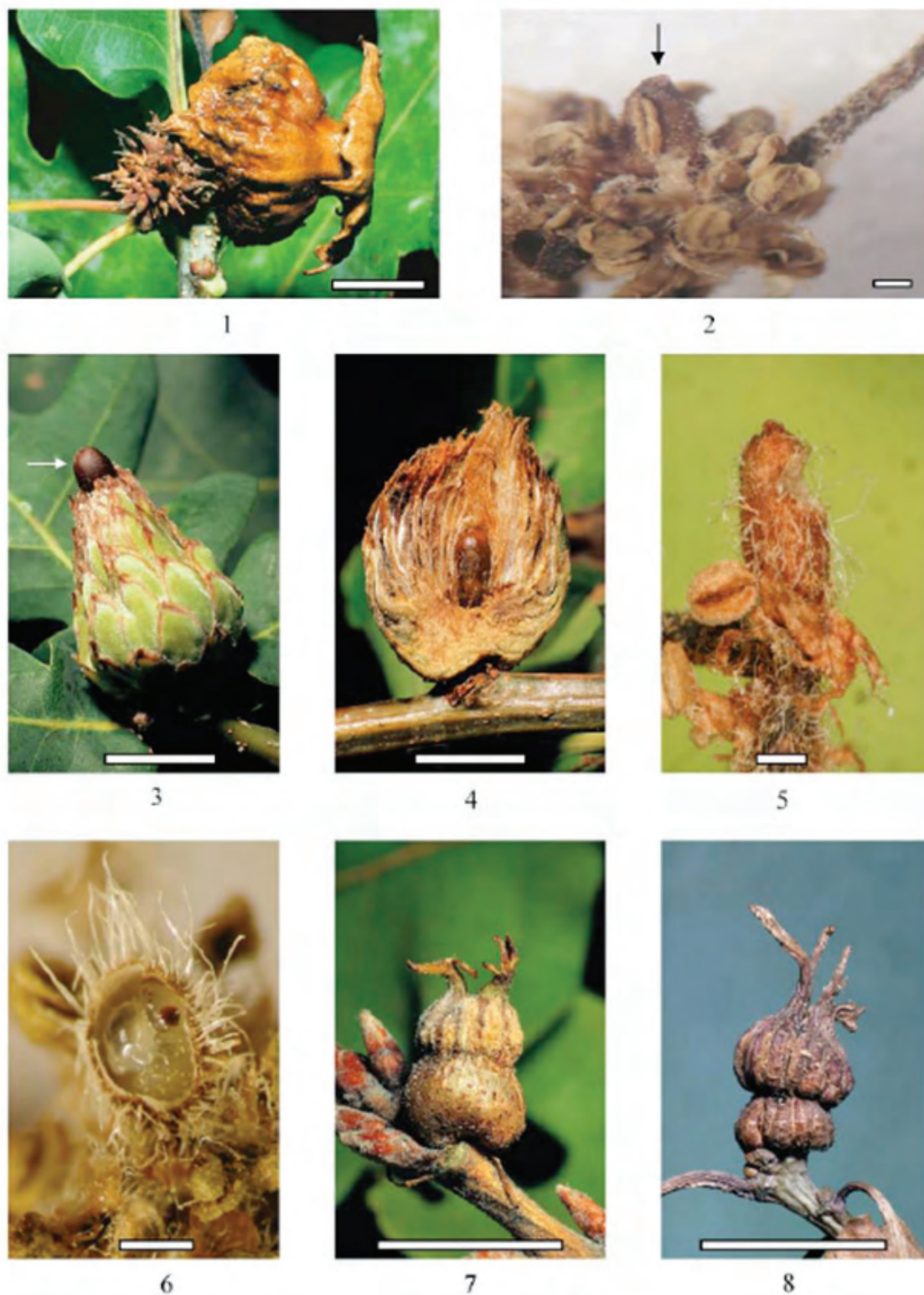
**Plate 4.** 1–2. *Andricus corruptrix* asexual galls (scale bar 1 cm) ©GyCs. 3–4. *Andricus corruptrix* sexual galls (scale bar 1 cm) ©GyCs, R F. 5–6. *Andricus crispator* sexual galls (scale bar 1 cm) ©GyCs. 7. *Andricus cryptobius* sexual gall (scale bar 1 cm) ©GyCs.



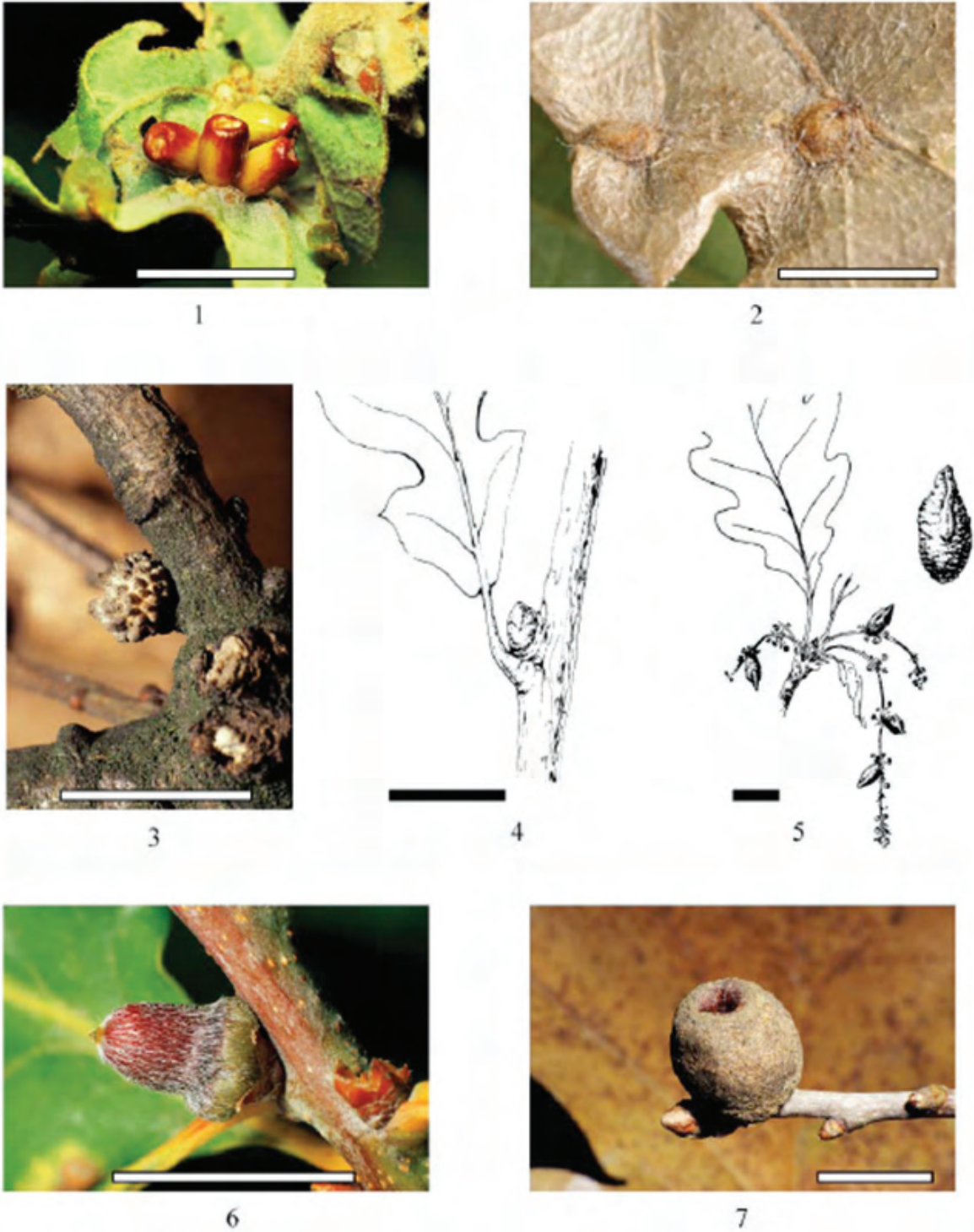


**Plate 5.** 1–4. *Andricus curtisii* asexual galls (scale bars 1 cm) 1 shows mature galls on *Q. infectoria* from southern Anatolia, with (inset) colour patterns seen in immature galls. 2–4 show galls on the same oak in northwestern Iran, immature in 2 and mature in 3–4 ©GS, MT, MT, MT 5–6. *Andricus curvator* asexual gall (scale bars 1 cm) ©JLNA, GyCs. 7–9. *Andricus curvator* sexual galls (scale bars 1 cm), opened in 9 to show the airspace around the larval chamber ©GyCs, GyCs, JLNA. 10–11. *Andricus cydoniae* sexual gall (scale bar 1 cm), opened to show multiple larval cells in 8. ©GyCs.



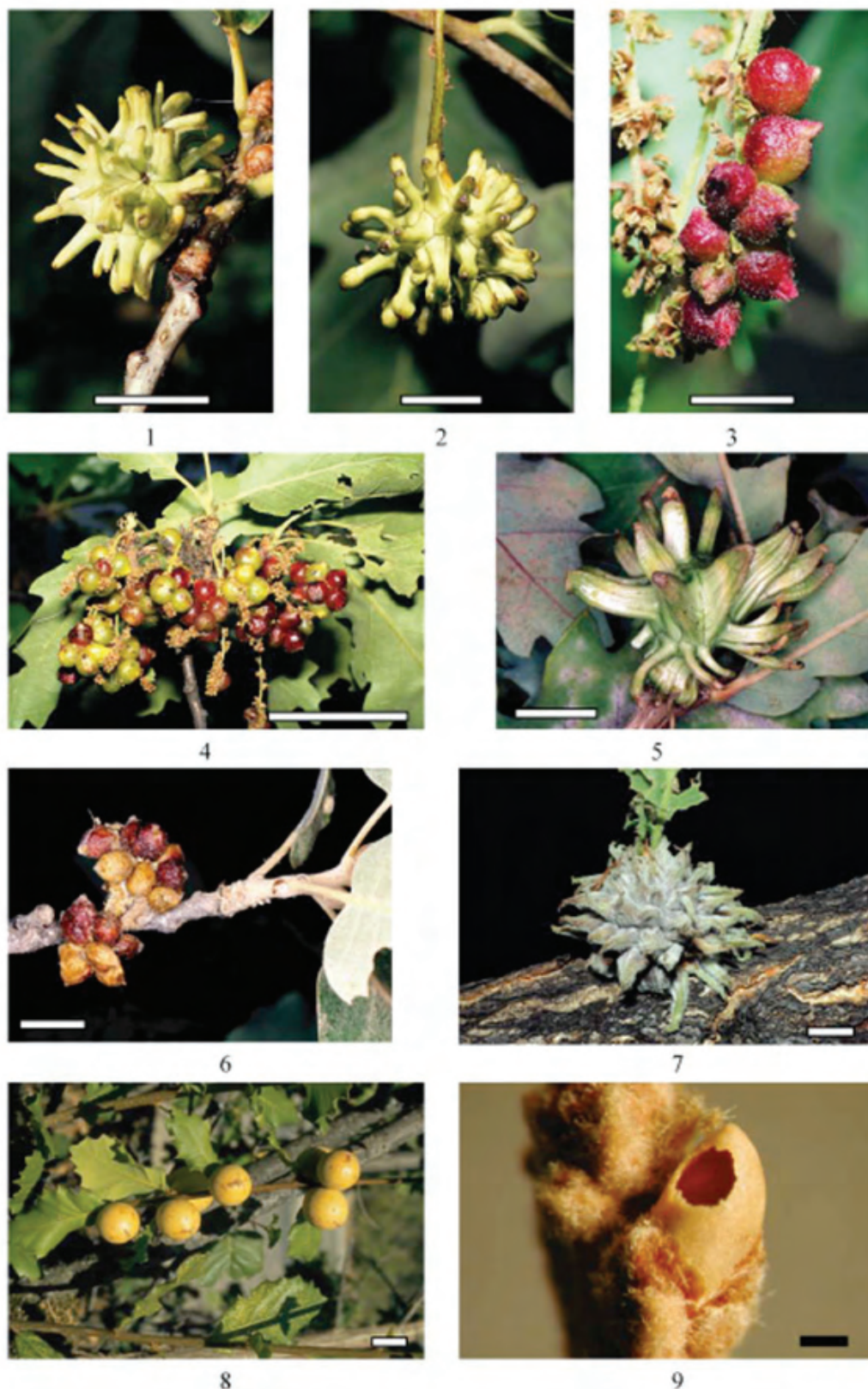


**Plate 6.** 1. *Andricus dentimitratus* asexual gall (scale bar 1 cm) ©GyCs. 2. *Andricus floridus* sexual gall (arrowed: scale bar 1 mm) ©JP. 3–4. *Andricus foecundatrix* asexual gall, dissected to show the inner larval chamber in 4, and with the larval chamber (arrowed) on the point of release in 3 (scale bar 1 cm) ©GyCs. 5–6. *Andricus foecundatrix* sexual gall, opened to show the gallwasp pupa within (scale bar 1 mm) ©GyCs, JLNA. 7–8. *Andricus galeatus* asexual gall (scale bar 1 cm) ©GyCs.

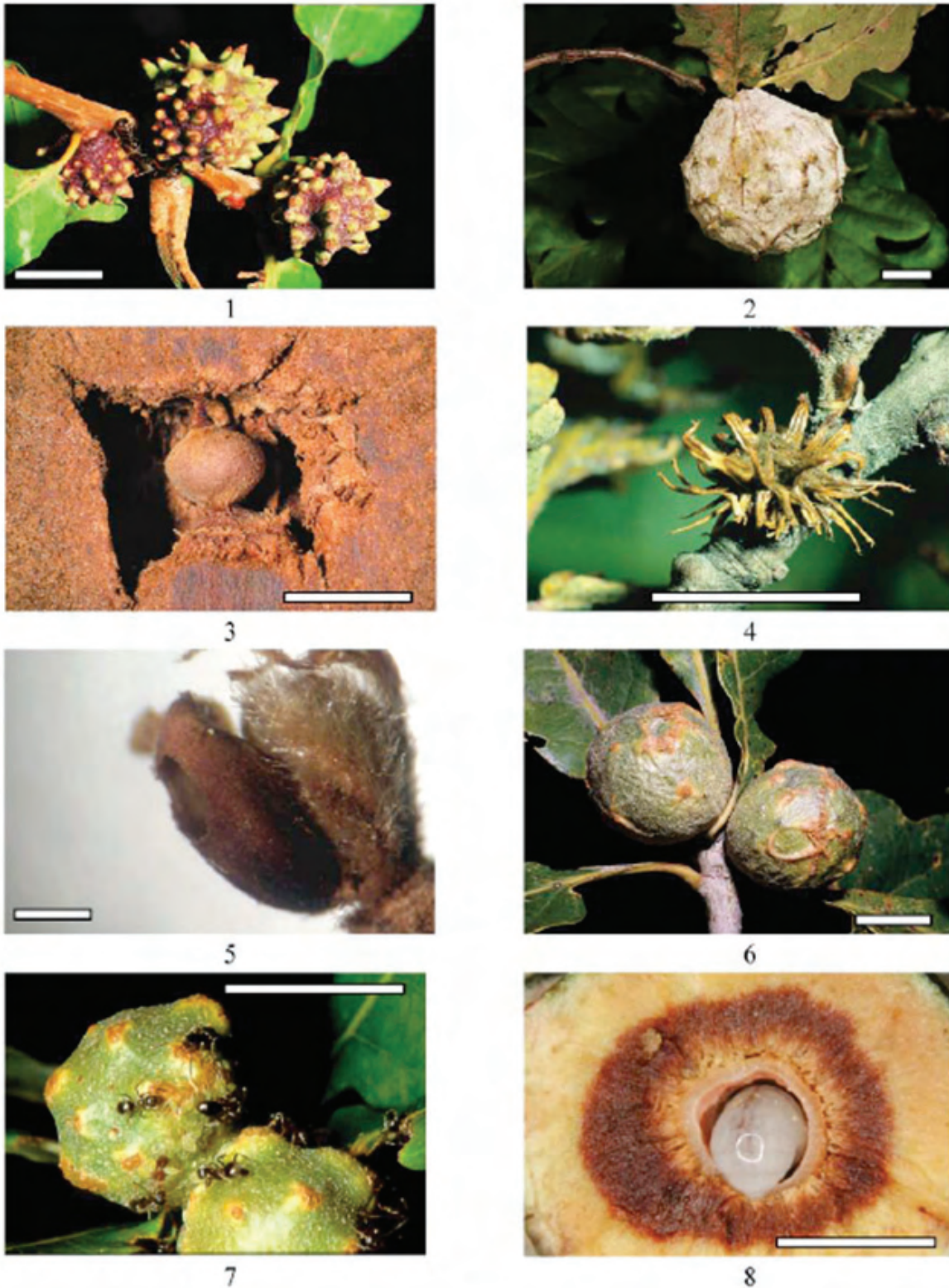


**Plate 7.** 1. *Andricus gallaeurnaeformis* asexual galls (scale bar 1 cm) ©GyCs. 2. *Andricus gallaeurnaeformis* sexual gall (scale bar 1 cm) ©GyCs. 3. *Andricus gemmeus* asexual gall (scale bar 1 cm) ©GyCs. 4. *Andricus gemmeus* sexual gall (scale bar 1 cm) ©BA. 5. *Andricus glandulae* sexual gall (scale bar 1 mm) ©BA. 6. *Andricus glandulae* asexual gall (scale bar 1 cm) ©GyCs. 7. *Andricus glutinosus* asexual gall (scale bar 1 cm) ©GyCs.



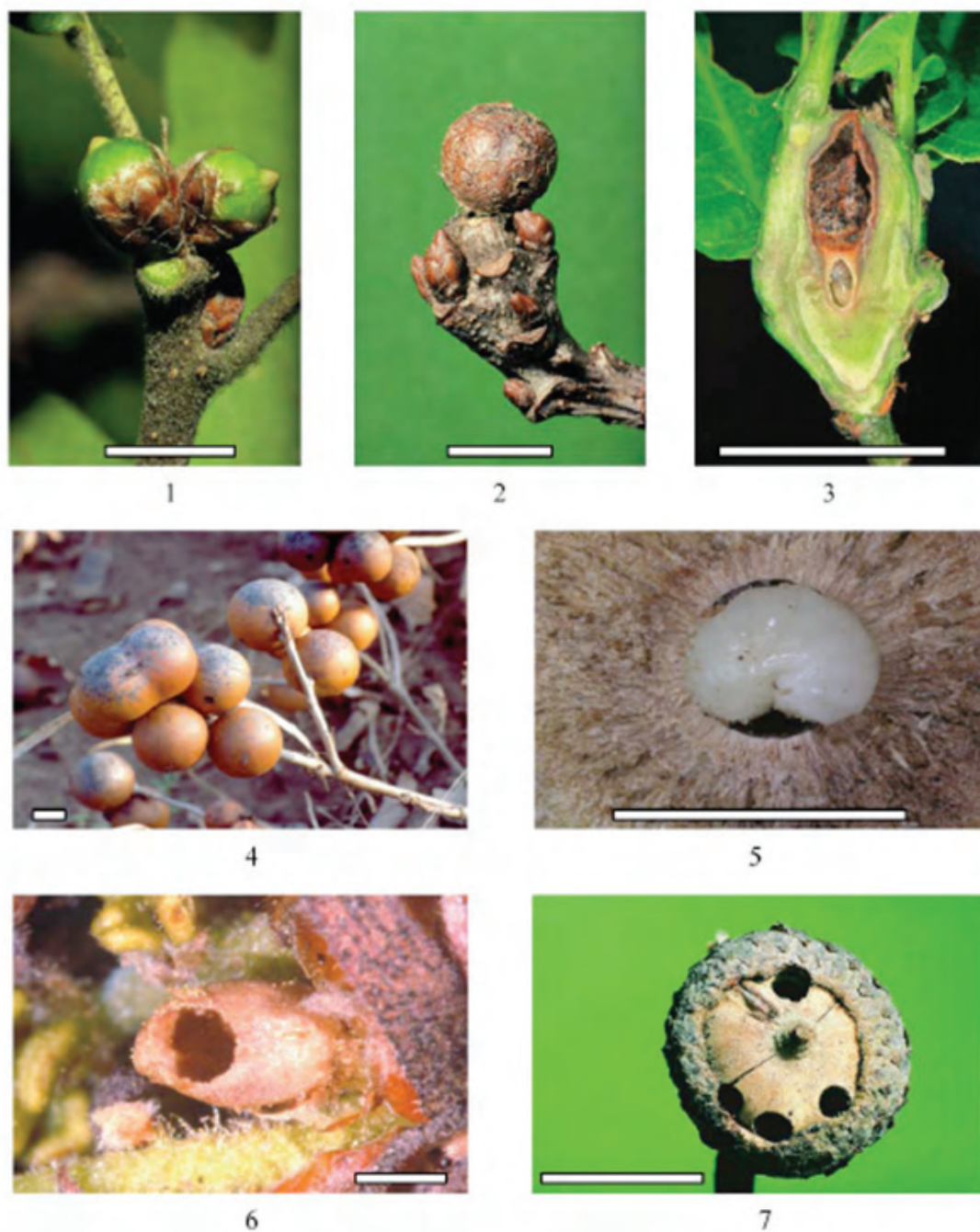


**Plate 8.** 1–2. *Andricus grossulariae* asexual galls on *Quercus robur* bud (1) and catkin (2) (scale bars 1 cm) ©GyCs. 3–4. *Andricus grossulariae* sexual galls on *Quercus cerris* (scale bars 1 cm, 5 cm) ©GyCs. 5. *Andricus grossulariae* asexual gall on *Quercus infectoria* (scale bar 1 cm) ©GS. 6. *Andricus grossulariae* sexual gall on *Quercus brantii* (scale bar 1 cm) ©GS. 7. *Andricus hartigi* asexual gall (scale bar 1 cm) ©GyCs. 8. *Andricus hispanicus* asexual galls (scale bar 1 cm) ©JLNA. 9. *Andricus hispanicus* sexual gall (scale bar 1 mm) ©JLNA.



**Plate 9.** 1–3. *Andricus hungaricus* asexual gall, when young (1), mature (2) and opened to show the internal airspace around the larval chamber (3) (scale bars 1 cm) ©GyCs. 4. *Andricus hystrix* asexual gall (scale bar 5mm) ©GyCs. 5. *Andricus hystrix* sexual gall (scale bar 1mm) ©RF. 6–7. *Andricus infectorius* asexual galls (scale bars 1 cm) ©GyCs, GS. 8. *Andricus infectorius* asexual gall dissected to show the larval gallwasp within (scale bar 1 cm) ©GyCs.





**Plate 10.** 1. *Andricus inflator* asexual galls (scale bar 1 cm) ©GyCs. 2. Old *Andricus inflator* sexual gall on which an asexual gall of *Andricus lignicola* has developed (scale bar 1 cm) ©GyCs. 3. *Andricus inflator* sexual gall dissected to show the single larval chamber within (scale bar 1 cm) ©GyCs. 4. *Andricus kollari* asexual galls (scale bar 1 cm) ©GyCs. 5. *Andricus kollari* asexual gall dissected to show the larval gallwasp within (scale bar 1 cm) ©GyCs. 6. *Andricus kollari* sexual galls (scale bar 1mm) ©GyCs. 7. *Andricus legitimus* asexual gall (scale bar 1 cm) ©GyCs.



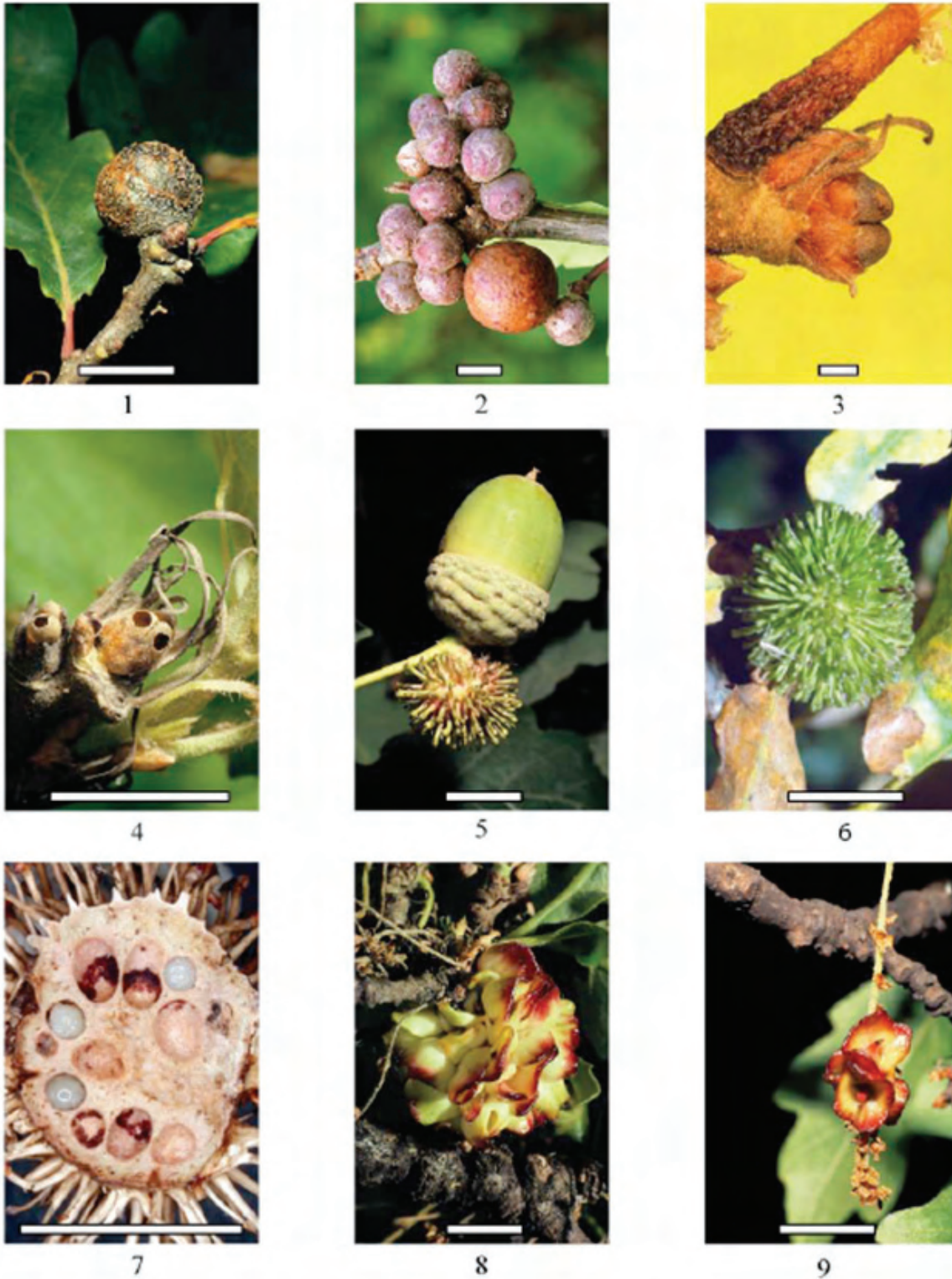
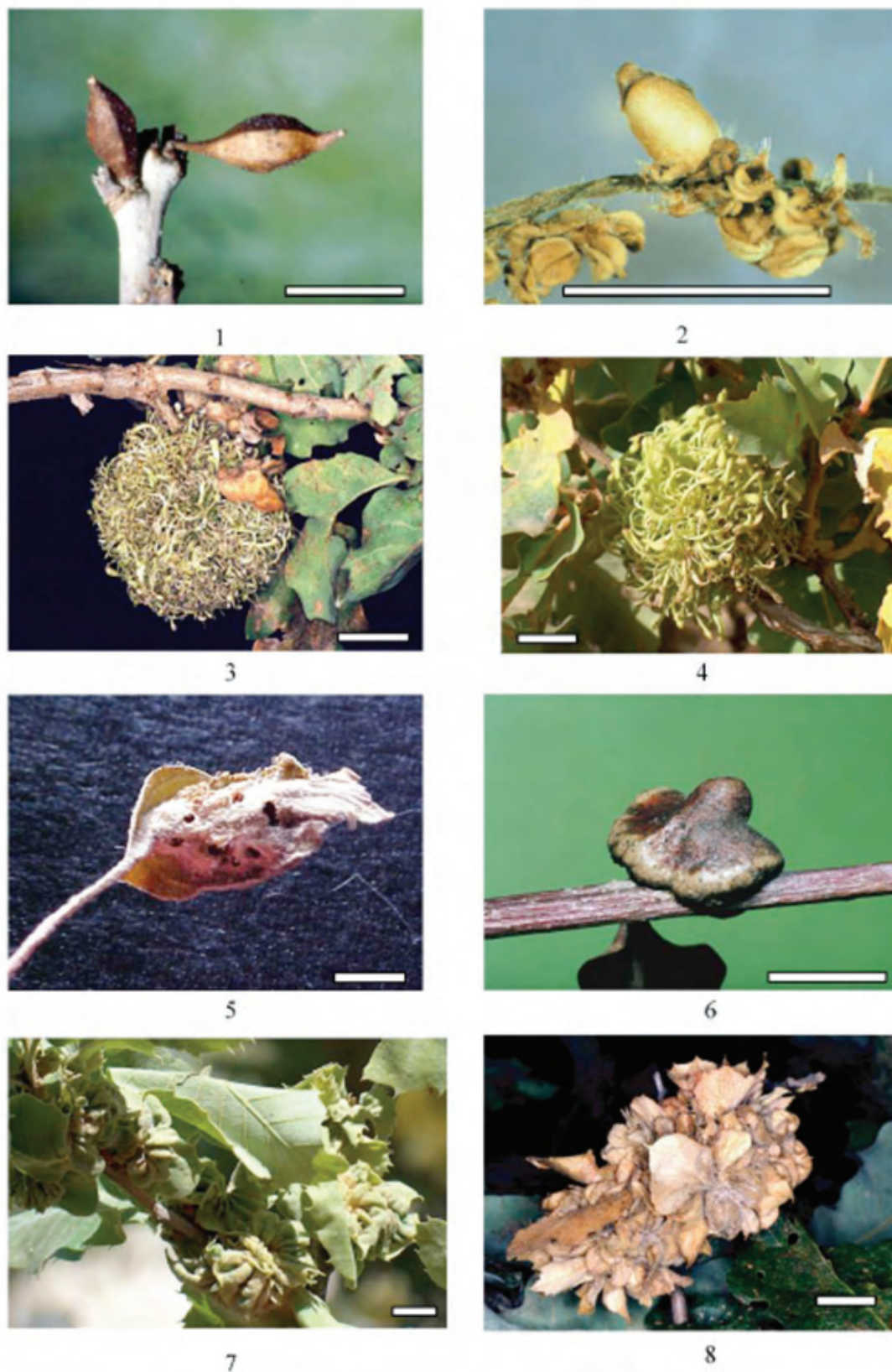
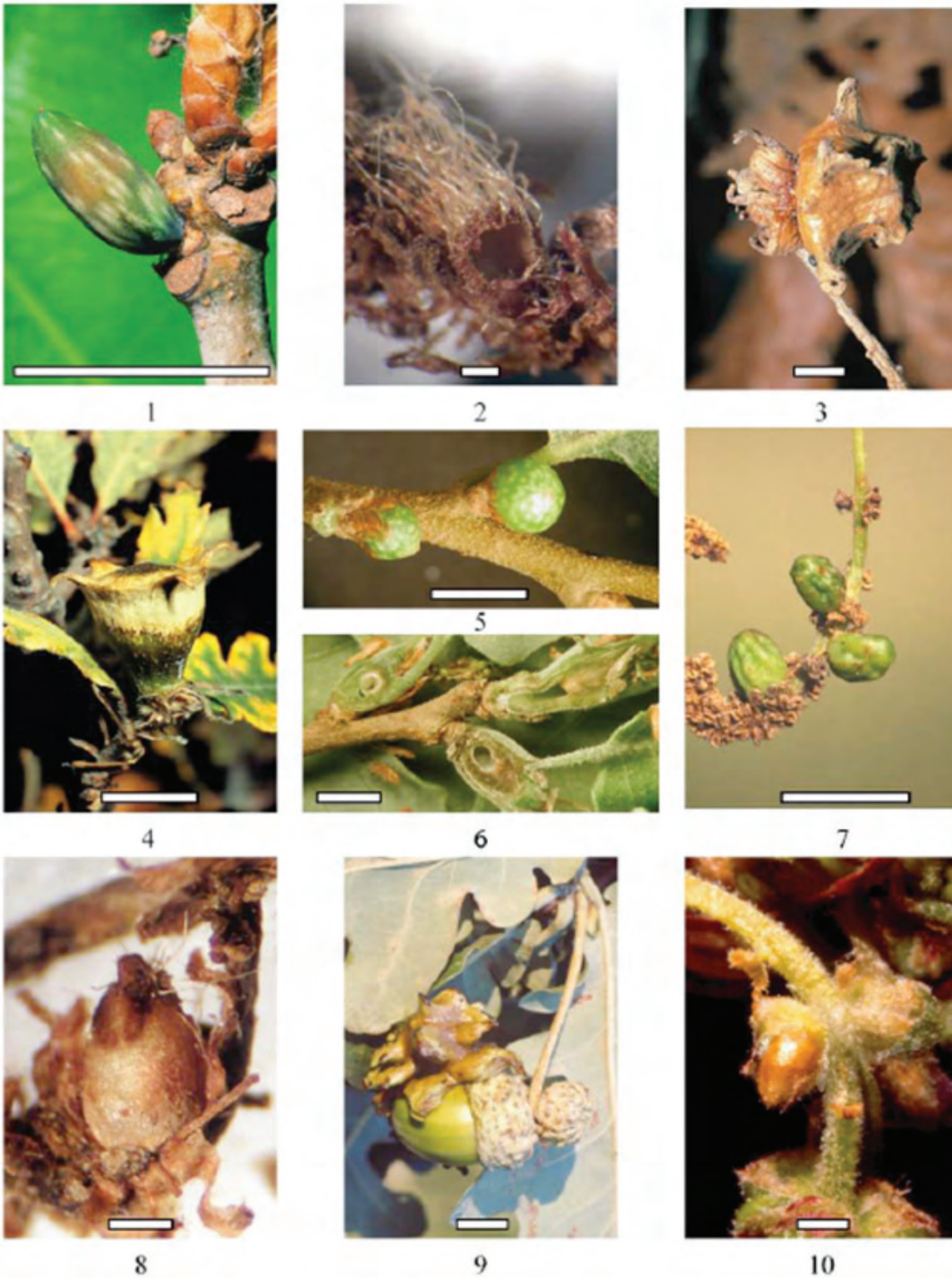


Plate 11. 1. *Andricus lignicolus* asexual gall (scale bar 1 cm) ©GyCs. 2. *Andricus lignicolus* asexual galls with a single *A. kollari* asexual gall (scale bar 1 cm) ©GyCs. 3–4. *Andricus lignicolus* sexual galls (scale bars 1mm, 1 cm) ©GyCs. 5–6. *Andricus lucidus* asexual galls on an acorn (5) and on a bud (6) (scale bars 1 cm) ©GyCs. 7. *Andricus lucidus* asexual gall dissected to show multiple larval chambers within (scale bar 1 cm) ©GyCs. 8–9. *Andricus lucidus* sexual galls (scale bars 1 cm) ©GyCs.



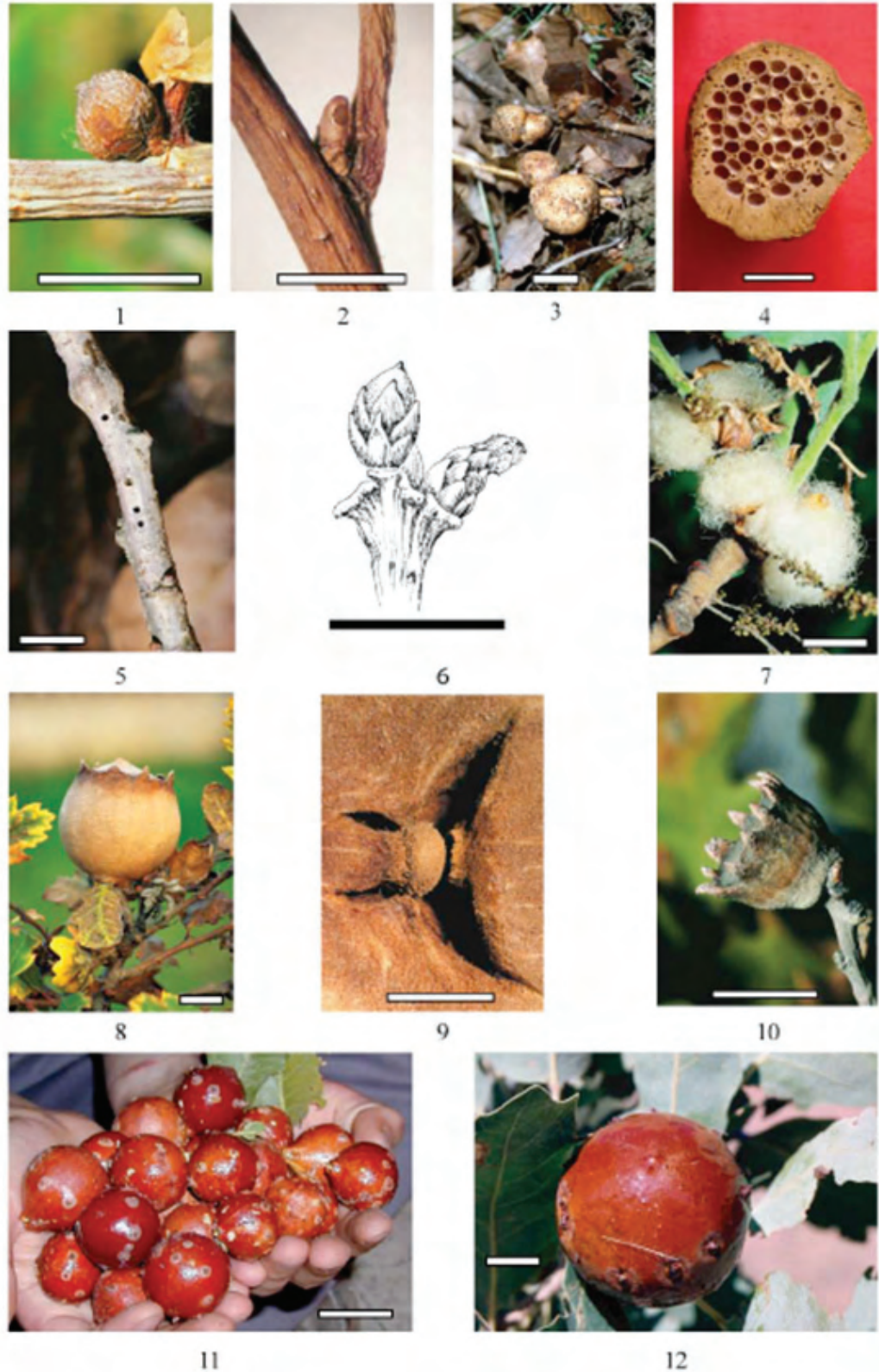
**Plate 12.** 1. *Andricus malpighii* asexual gall (scale bar 1 cm) ©GyCs. 2. *Andricus malpighii* sexual gall (scale bar 1 cm) ©RF. 3–4. *Andricus megalucidus* asexual galls (scale bars 1 cm) ©GS, MT. 5. *Andricus melikai* sexual gall (scale bar 1 cm) ©JP. 6. *Andricus mitratus* asexual gall (scale bar 1 cm) ©GyCs. 7–8. *Andricus multiplicatus* sexual galls on *Quercus brantii*, Iran (scale bars 1 cm) ©MT, GS.



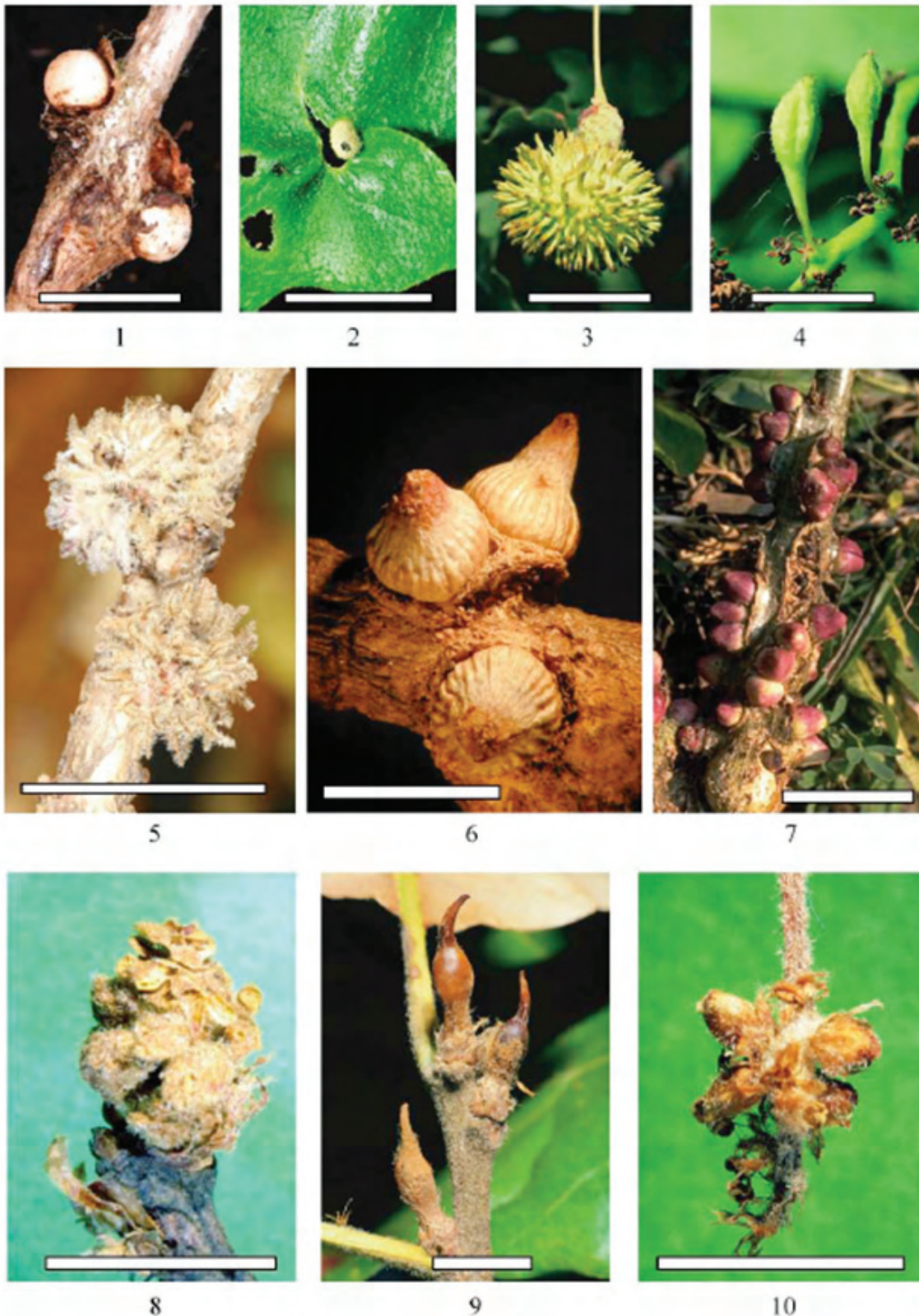


**Plate 13.** 1–2. *Andricus paradoxus* asexual and sexual gall (scale bars 1 cm, 1mm) ©GyCs, JP. 3. *Andricus pictus* asexual gall (scale bar 1 cm) ©GyCs. 4. *Andricus polycerus* asexual gall (scale bar 1 cm) ©GyCs. 5. *Andricus pseudoinflator* asexual gall (scale bar 1 cm) ©JLNA. 6. *Andricus pseudoinflator* sexual gall dissected to show internal structure (scale bar 1 cm) ©JLNA. 7–8. *Andricus quadrilineatus* asexual (7) and sexual (8) galls (scale bars 1 cm and 1mm) ©JLNA. 9. *Andricus quercuscalicis* asexual gall (scale bar 1 cm) ©GyCs 10. *Andricus quercuscalicis* sexual galls (scale bar 1mm) ©GyCs.



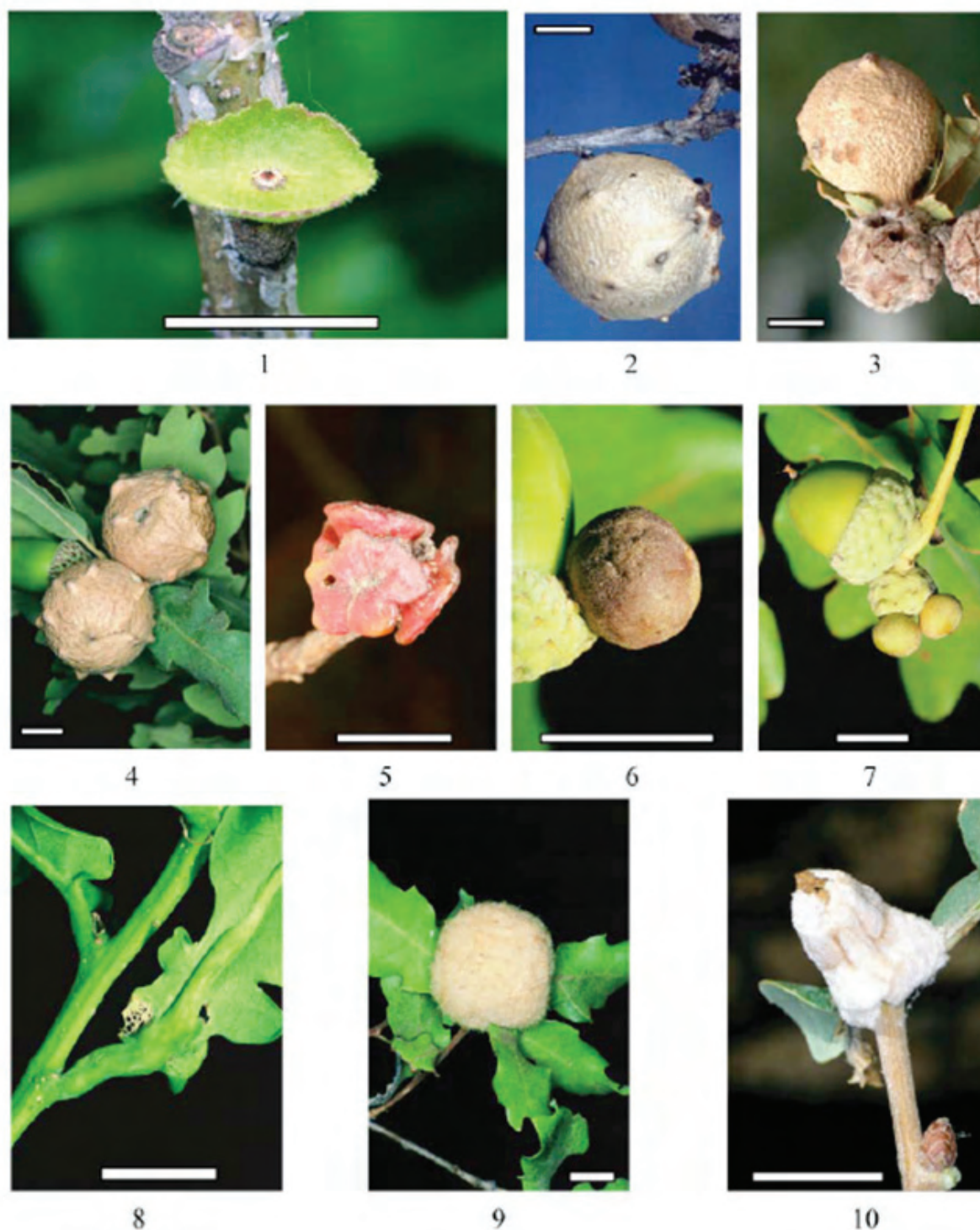


**Plate 14.** 1. *Andricus quercuscorticis* asexual gall (scale bar 1 cm) ©GyCs. 2. *Andricus quercuscorticis* sexual gall (scale bar 1 cm) ©JP. 3–4. *Andricus quercusradicis* asexual galls (scale bars 1 cm) ©JLNA, GyCs. 5. *Andricus quercusradicis* sexual galls (scale bar 1 cm) ©GyCs. 6. *Andricus quercusramuli* asexual gall (scale bar 1 cm) ©BA. 7. *Andricus quercusramuli* sexual galls (scale bar 1 cm) ©GyCs. 8–9. *Andricus quercustozae* asexual gall, opened to show the internal airspace around the larval cell in 9 (scale bars 1 cm) ©GyCs. 10. *Andricus quercustozae* asexual gall, stunted by inquiline or parasitoid attack (scale bar 1 cm) ©GyCs. 11–12. *Andricus quercustozae* f. *insana* asexual galls (scale bars 5 cm, 1 cm) ©GS.



**Plate 15** 1. *Andricus rhyzomae* asexual gall (scale bar 1 cm) ©GyCs. 2. *Andricus schroeckingeri* sexual gall (scale bar 1 cm) ©GyCs. 3. *Andricus seckendorffi* asexual gall (scale bar 2 cm) ©GyCs. 4. *Andricus seminationis* asexual gall (scale bar 1 cm) ©GyCs. 5. *Andricus serotinus* asexual gall (scale bar 1 cm) ©GyCs. 6–7. *Andricus sieboldi* asexual galls (scale bars 1 cm and 5 cm) ©GS, JLNA. 8. *Andricus singularis* sexual gall (scale bar 1 cm) ©GyCs. 9. *Andricus solitarius* asexual gall (scale bar 1 cm) ©GyCs. 10. *Andricus solitarius* sexual gall (scale bar 1 cm) ©GyCs.





**Plate 16.** 1. *Andricus stefanii* asexual gall (scale bar 1 cm) ©GyCs. 2–4. *Andricus sternlichti* asexual galls (uppermost gall in 3) (scale bars 1 cm) ©JP, MT, GS 5. *Andricus subterraneus* asexual gall (scale bar 1 cm) ©GyCs. 6–7. *Andricus superfetationis* asexual gall (scale bar 1 cm) ©GyCs. 8. *Andricus testaceipes* sexual gall (scale bar 1 cm) ©GyCs. 9. *Andricus theophrastea* asexual gall (scale bar 1 cm) ©GS. 10. *Andricus tomentosus* asexual gall (scale bar 1 cm) ©GS.





**Plate 17.** 1. *Andricus truncicolus* asexual gall (scale bar 1 cm) ©GyCs. 2. *Andricus vindobonensis* sexual galls (scale bar 1 cm) ©GyCs. 3–4. *Aphelonyx cerricola* asexual galls, with a single gall opened to show the central larval chamber in 4 (scale bars 1 cm) ©GyCs. 5–6. *Aphelonyx persica* asexual galls (scale bar 1 cm) ©GS. 7. *Biorhiza pallida* asexual gall (scale bar 1 cm) ©JP. 8–9. *Biorhiza pallida* sexual gall, opened in 9 to show the larval chambers (scale bars 1 cm) ©GyCs.



**Plate 18.** 1–2. *Callirhytis glandium* asexual galls in an acorn of *Quercus cerris* (scale bar 1 cm) ©GyCs. 3. *Callirhytis glandium* asexual galls in an acorn of *Quercus robur* (scale bar 1 cm) ©GyCs. 4. *Callirhytis glandium* sexual gall (scale bar 1 cm) ©GyCs. 5. *Callirhytis rufescens* asexual galls (scale bar 1 cm) ©JLNA. 6–7. *Callirhytis rufescens* sexual galls (scale bar 1 cm) ©JLNA.





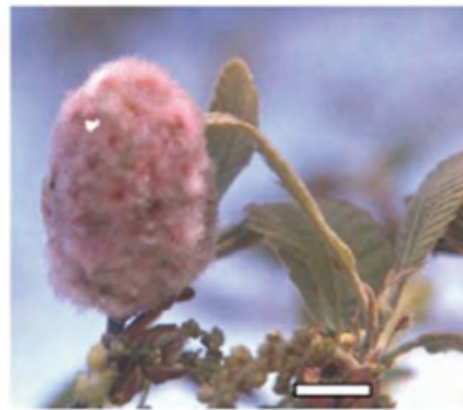
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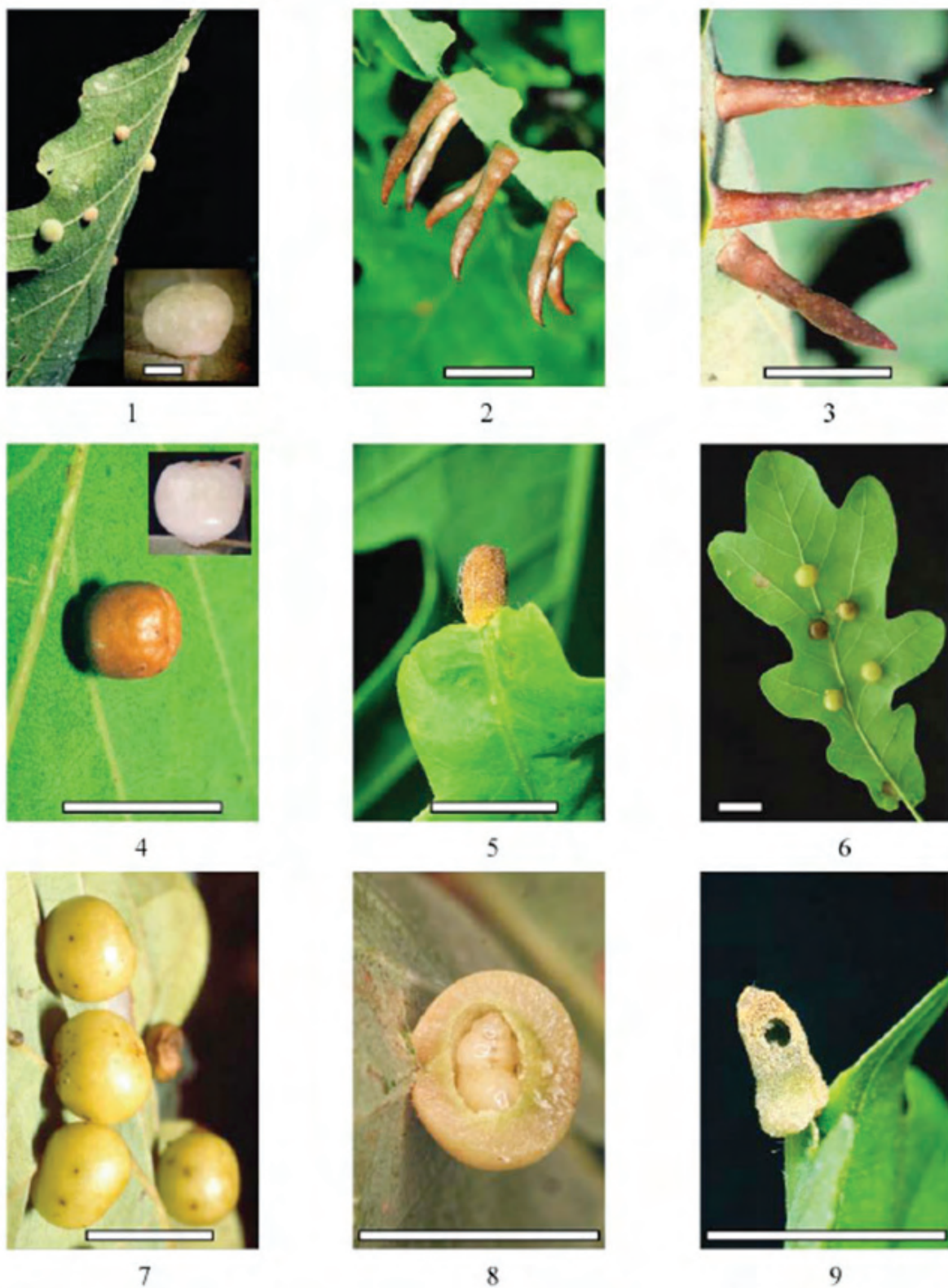
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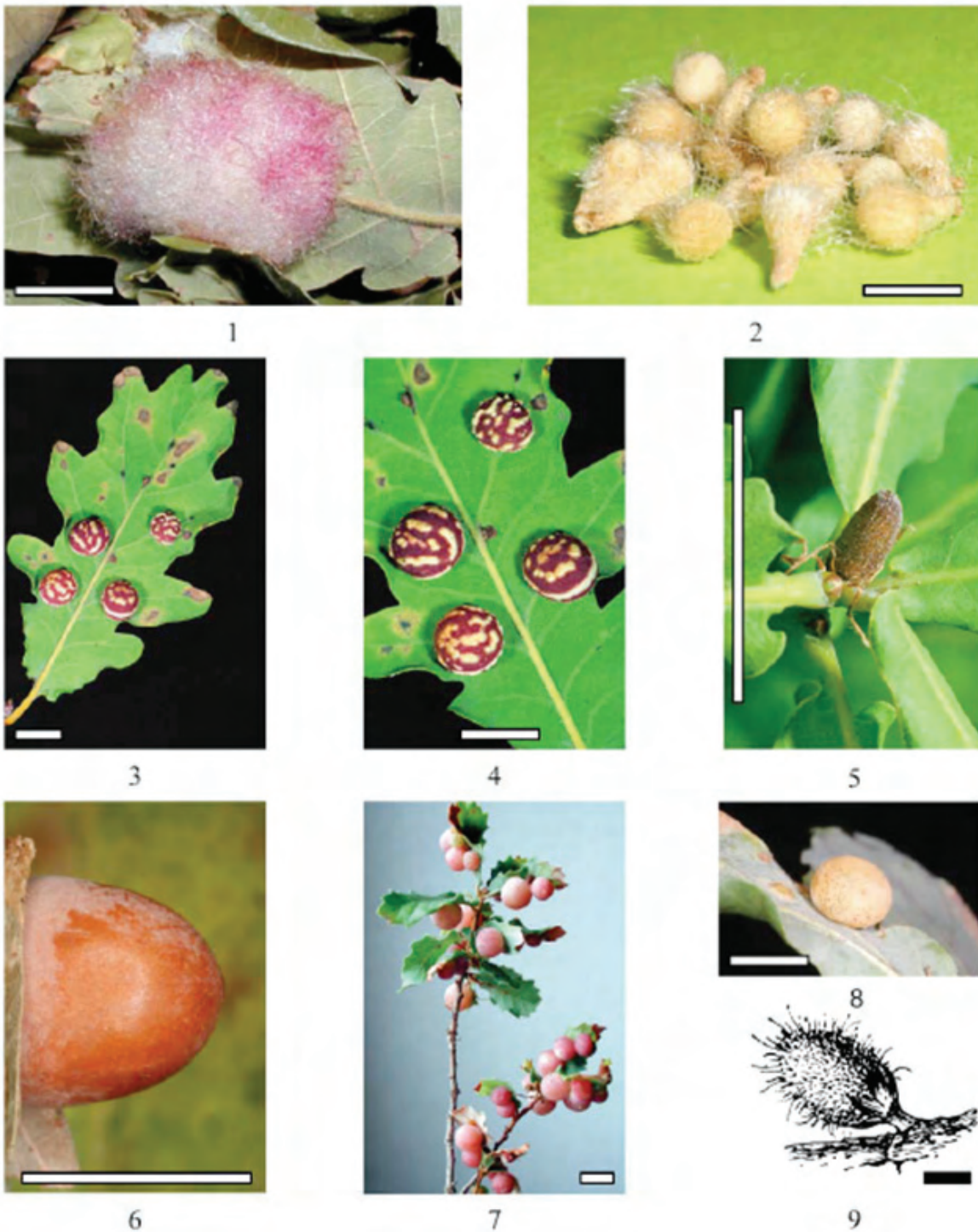
8

**Plate 19.** 1–2. *Chilaspis israeli* asexual galls (scale bars 1 cm) ©GS. 3–4. *Chilaspis israeli* sexual galls (scale bars 1 cm) ©MT. 5–6. *Chilaspis mayri* sexual galls (scale bars 1 cm) ©GyCs. 7. *Chilaspis nitida* asexual galls (scale bar 1 cm) ©GyCs. 8. *Chilaspis nitida* sexual galls (scale bar 1 cm) ©GyCs.



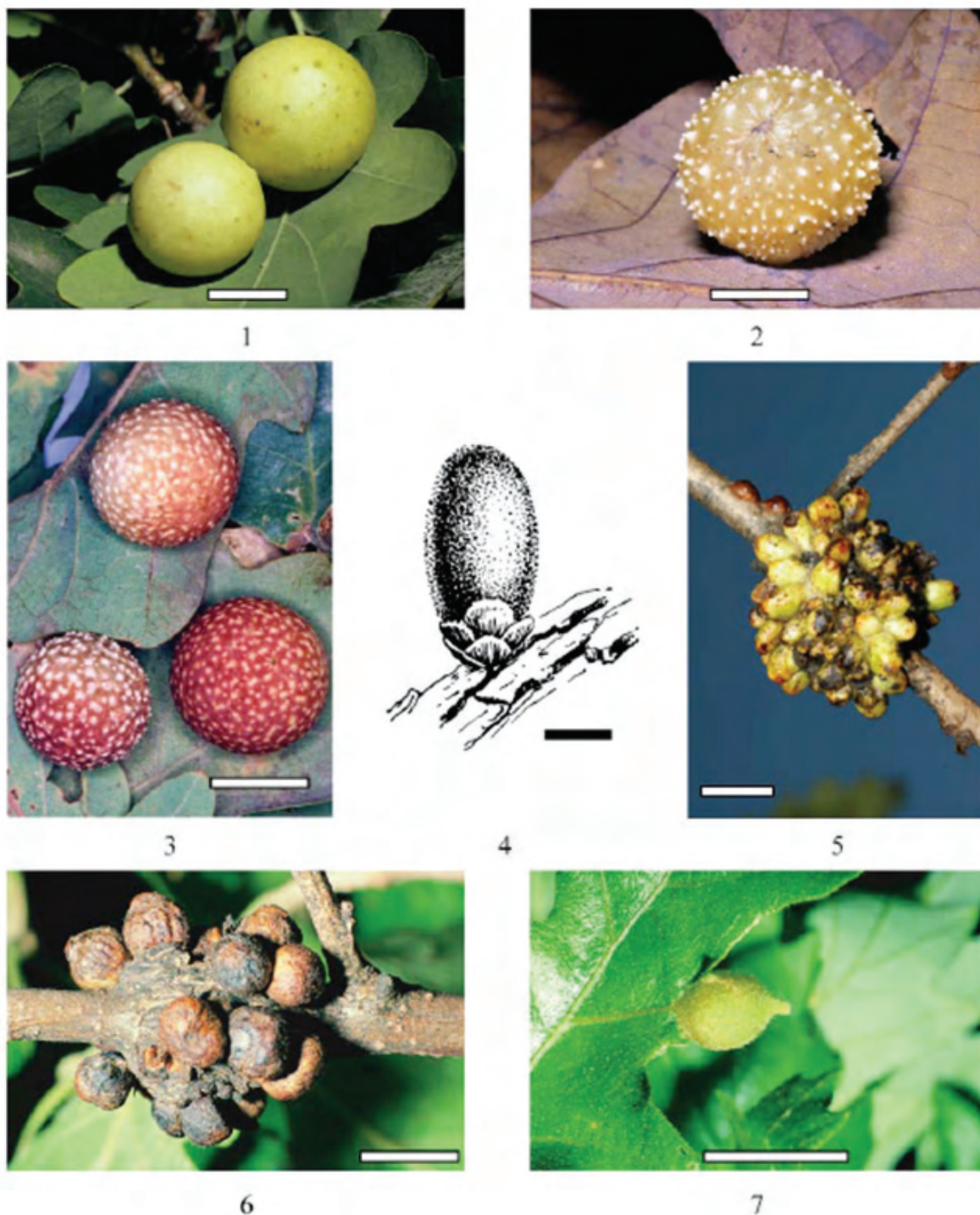


**Plate 20.** 1. *Cynips agama* asexual galls (scale bar in inset 1mm) ©GS. 2–3. *Cynips cornifex* asexual gall (scale bar 1 cm) ©GyCs. 4. *Cynips disticha* asexual gall (scale bar 1 cm) when mature and (inset) when young ©GyCs, GS. 5. *Cynips disticha* sexual gall (scale bar 1 cm) ©GyCs. 6–8. *Cynips divisa* asexual gall (scale bar 1 cm) ©GyCs. 9. *Cynips divisa* sexual gall (scale bar 1 cm) ©GyCs.

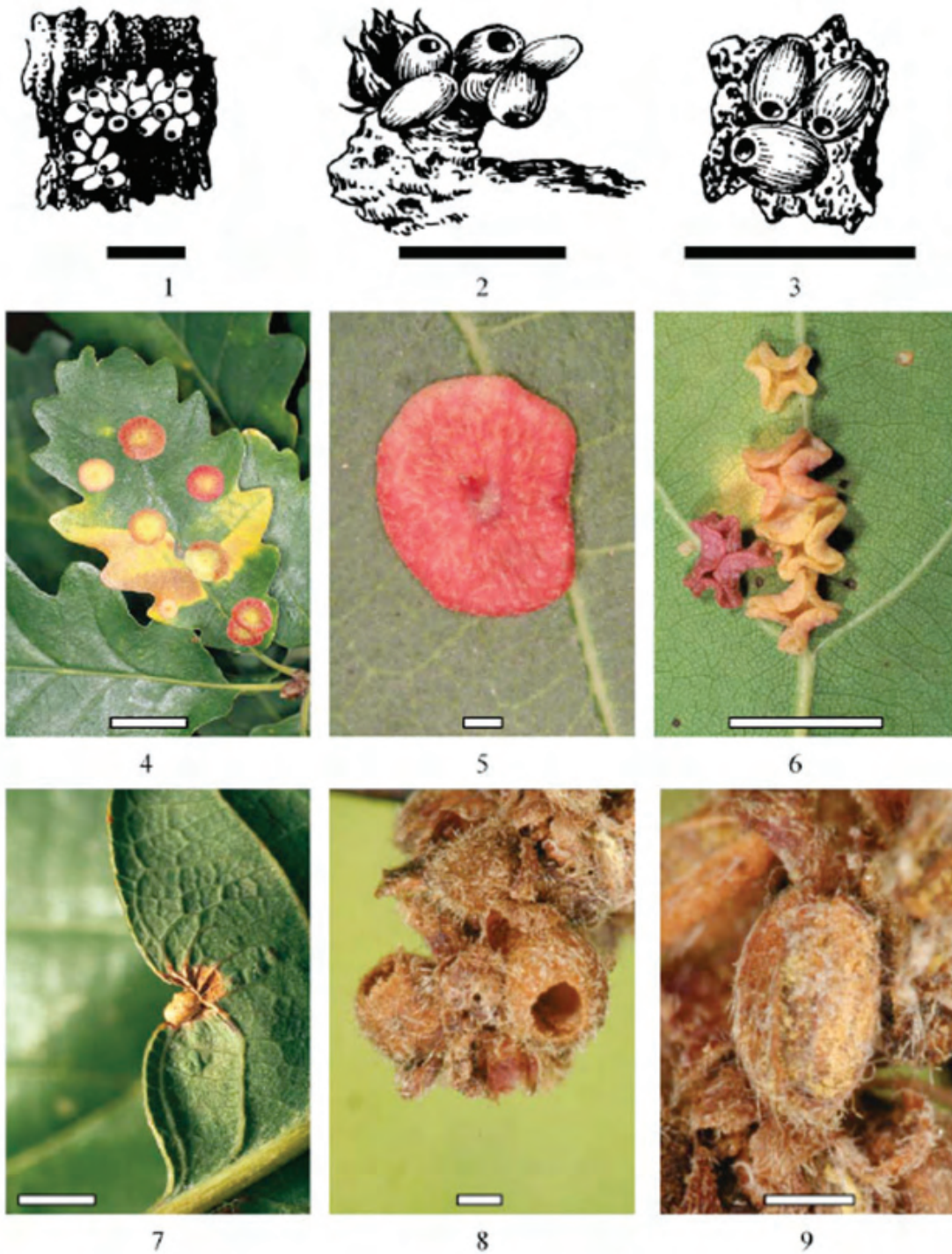


**Plate 21.** 1–2. *Cynips korsakovi* asexual gall (scale bars 1 cm) ©GS, GyCs. In 2 the individual cells that make up the aggregated structure shown in 1 have been separated. 3–4. *Cynips longiventris* asexual galls (scale bars 1 cm) ©GyCs. 5. *Cynips longiventris* sexual gall (scale bar 1 cm) ©GyCs. 6. *Cynips loricatus* asexual gall (scale bar 1 cm) ©GyCs. 7–8. *Cynips quercus* asexual galls (scale bars 1 cm) ©JLNA, GyCs. 9. *Cynips quercus* sexual gall (scale bar 1mm) ©BA.



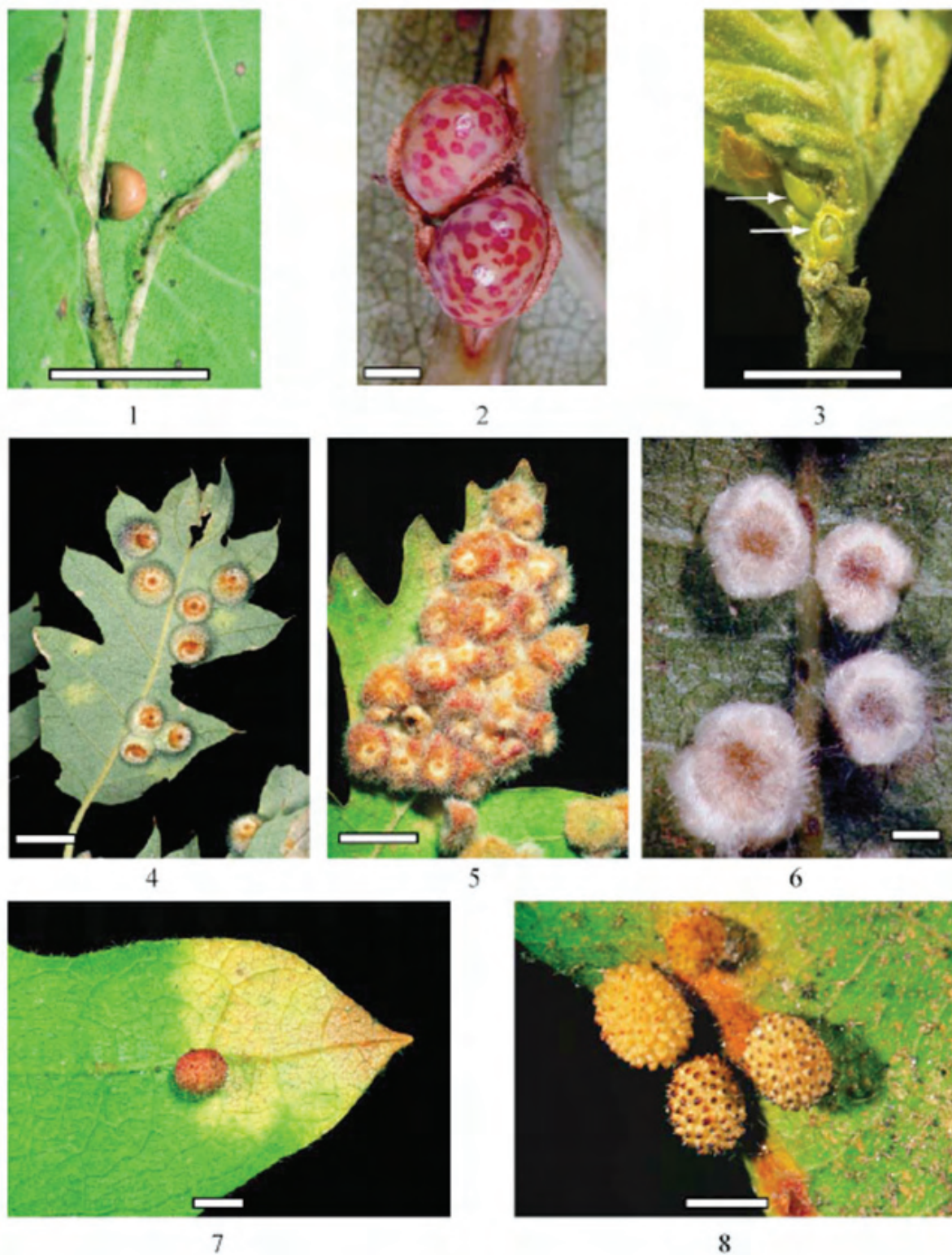


**Plate 22.** 1. *Cynips quercusfolii* asexual galls on *Quercus robur* (scale bar 1 cm) ©GyCs. 2. *Cynips quercusfolii* asexual galls on *Quercus petraea* (scale bar 1 cm) ©GyCs. 3. *Cynips quercusfolii* asexual galls on *Quercus infectoria* (scale bar 1 cm) ©GS. 4. *Cynips quercusfolii* sexual gall (scale bar 1mm) ©BA. 5. *Dryocosmus cerriphilus* asexual gall (scale bar 1 cm) ©GyCs. 6. Old asexual gall of *Dryocosmus cerriphilus* (scale bar 1 cm) ©GyCs. 7. *Dryocosmus cerriphilus* sexual gall (scale bar 1 cm) ©GyCs.

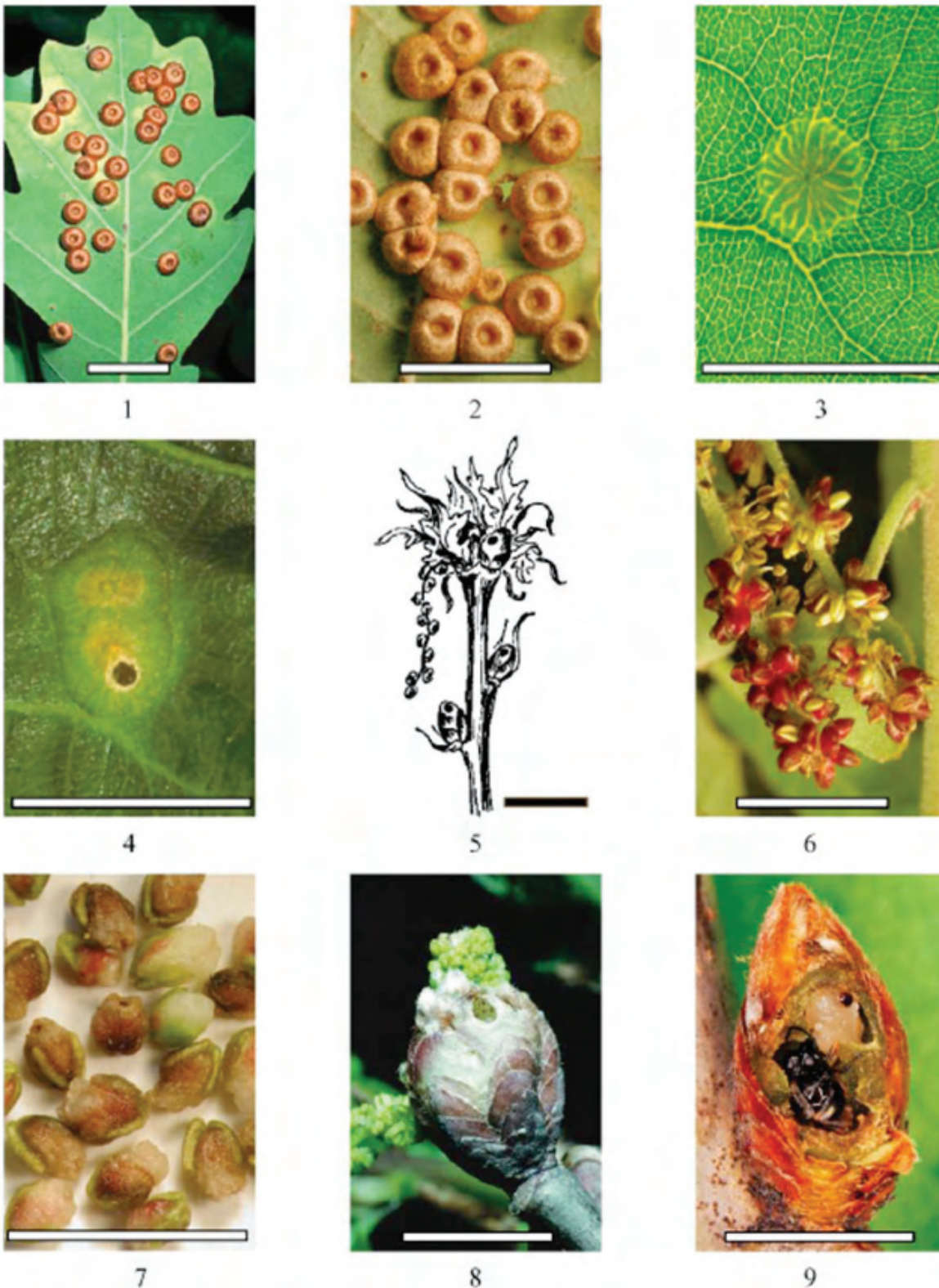


**Plate 23.** 1–3. *Neuroterus aggregatus* sexual galls (scale bar 1 cm) ©BA. 4–5. *Neuroterus albipes* asexual galls (scale bars 1 cm, 1 mm) ©GyCs. 6. *Neuroterus albipes f. reflexus* sexual gall (scale bar 1 cm) ©GyCs. 7. *Neuroterus albipes* sexual gall (scale bar 1 cm) ©GyCs. 8–9. *Neuroterus ambrusi* sexual galls (scale bar 1 mm) ©GyCs.



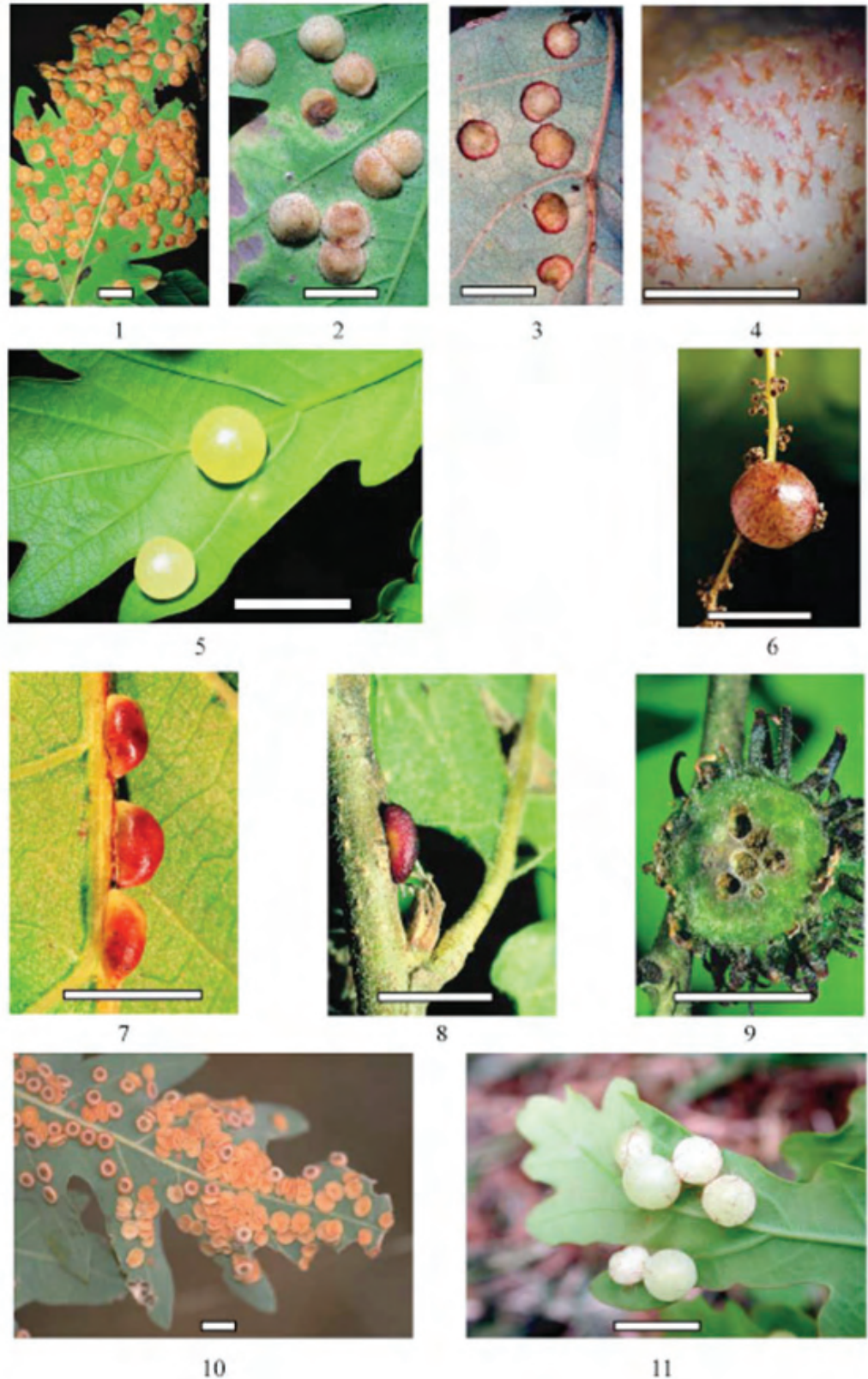


**Plate 24.** 1–2. *Neuroterus anthracinus* asexual galls (scale bar 1 cm, 1mm) ©GyCs, GS. 3. *Neuroterus anthracinus* sexual gall (arrowed; scale bar 1 cm) ©JLNA. 4–6. *Neuroterus lanuginosus* asexual galls (scale bars 1 cm, 1 cm, 1mm) ©GS, GyCs, GS. 7–8. *Neuroterus minutulus* asexual galls (scale bar 1mm) ©GyCs.

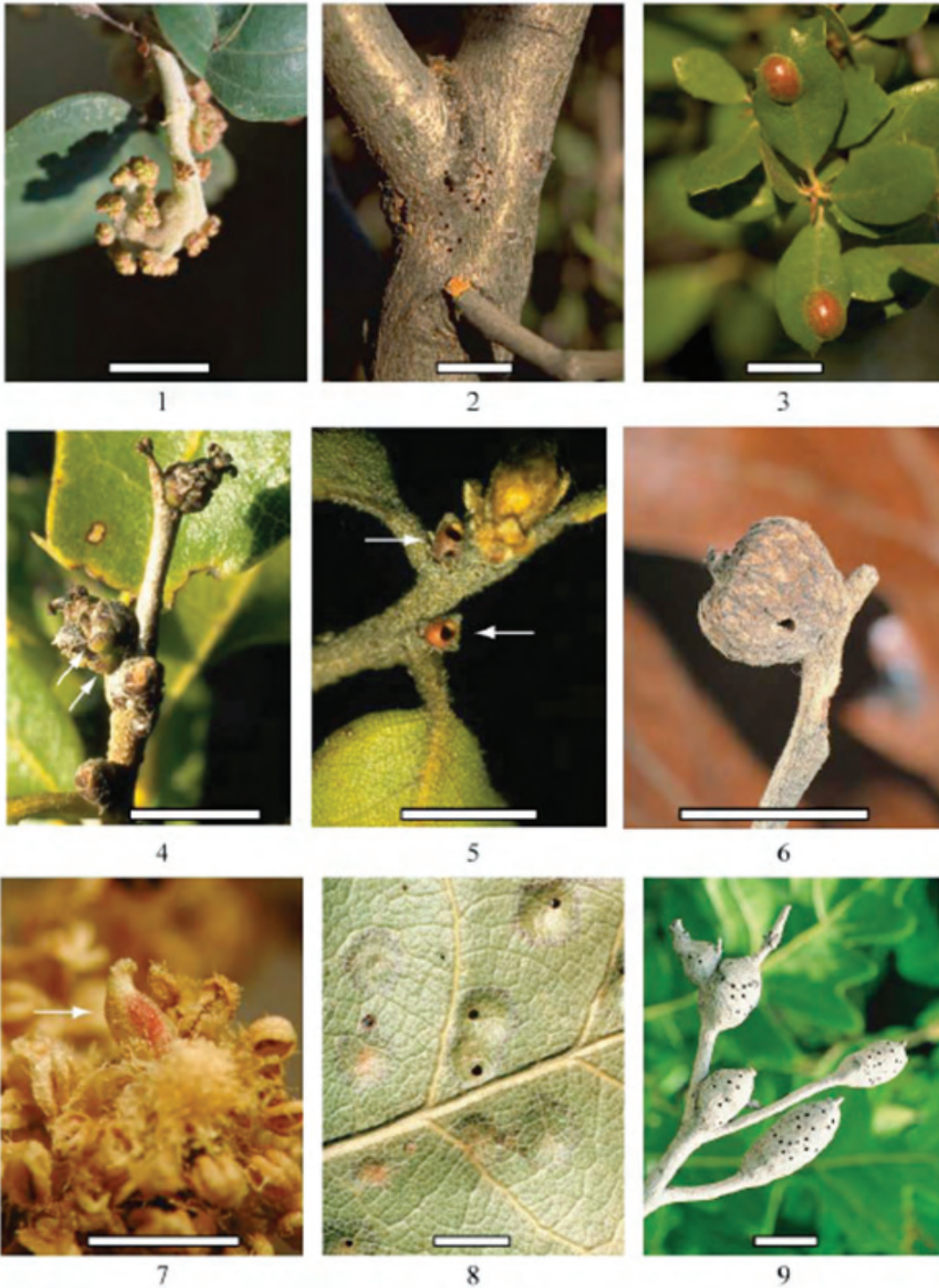


**Plate 25.** 1–2. *Neuroterus numismalis* asexual galls (scale bars 1 cm) ©GyCs. 3–4. *Neuroterus numismalis* sexual galls (scale bar 1 cm) ©GyCs. 5. *Neuroterus obtectus* sexual galls (scale bar 1 cm) ©BA. 6–7. *Neuroterus politus* asexual galls (scale bars 1 cm) ©JLNA. 8–9. *Neuroterus politus* sexual galls (scale bar 1 cm), in 9 opened to show multiple cells occupied by *Synergus inquilines* ©GyCs.



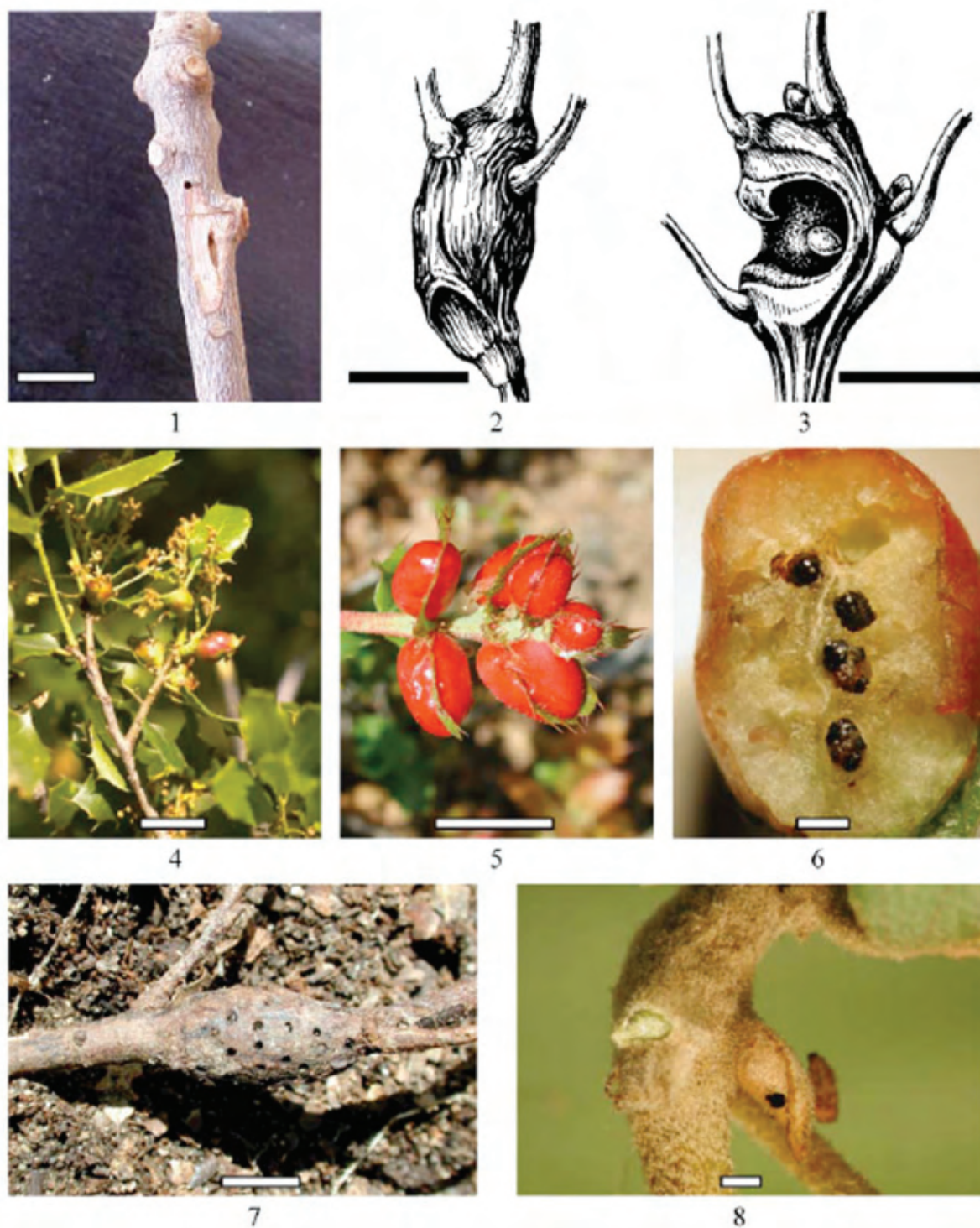


**Plate 26.** 1–3. *Neuroterus quercusbaccarum* asexual galls (scale bar 1 cm), with the reddish rim characteristic of galls on *Quercus infectoria* visible in 3. ©GyCs, GyCs, GS. 4. Close-up of the same gall showing stellate hairs (scale bar 1mm) ©GS. 5–6. *Neuroterus quercusbaccarum* sexual galls (scale bar 1 cm) ©GyCs. 7–8. *Neuroterus saliens* asexual galls (scale bar 1 cm) ©GyCs. 9. *Neuroterus saliens* sexual gall in a first year acorn of *Quercus cerris*, opened to show multiple larval chambers (scale bar 1 cm) ©GyCs. 10. *Neuroterus tricolor* asexual galls (scale bar 1 cm) ©JLNA. 11. *Neuroterus tricolor* sexual galls (scale bar 1 cm) ©JLNA.

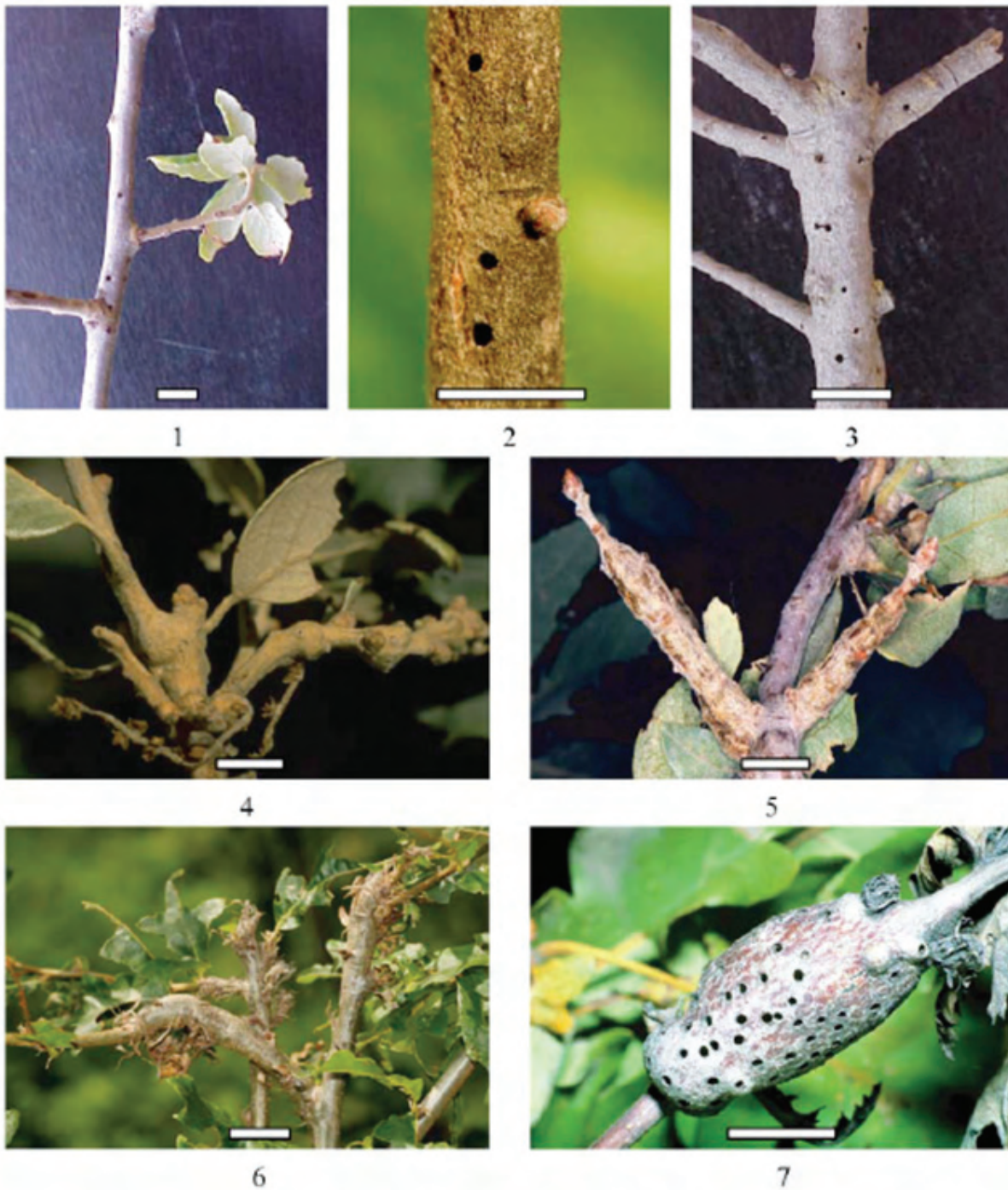


**Plate 27.** 1. *Plagiotrochus amenti* sexual gall (scale bar 1 cm) ©JLNA. 2. *Plagiotrochus australis* asexual galls (scale bar 1 cm) ©JLNA. 3. *Plagiotrochus australis* sexual galls (scale bar 1 cm) ©JLNA. 4–5. *Plagiotrochus britanniae* sexual galls (arrowed) on an acorn cup of *Q. coccifera* (4) and in an axillary bud (5) (scale bars 1 cm) ©JLNA. 6. *Plagiotrochus burnayi* asexual gall (scale bar 1 cm) ©GyCs. 7. *Plagiotrochus cardiguensis* sexual gall (arrowed: scale bar 1 cm) ©JLNA. 8. *Plagiotrochus coriaceus* asexual galls (scale bar 1 cm) ©GyCs. 9. *Plagiotrochus gallaeramulorum* asexual gall (scale bar 1 cm) ©GyCs.



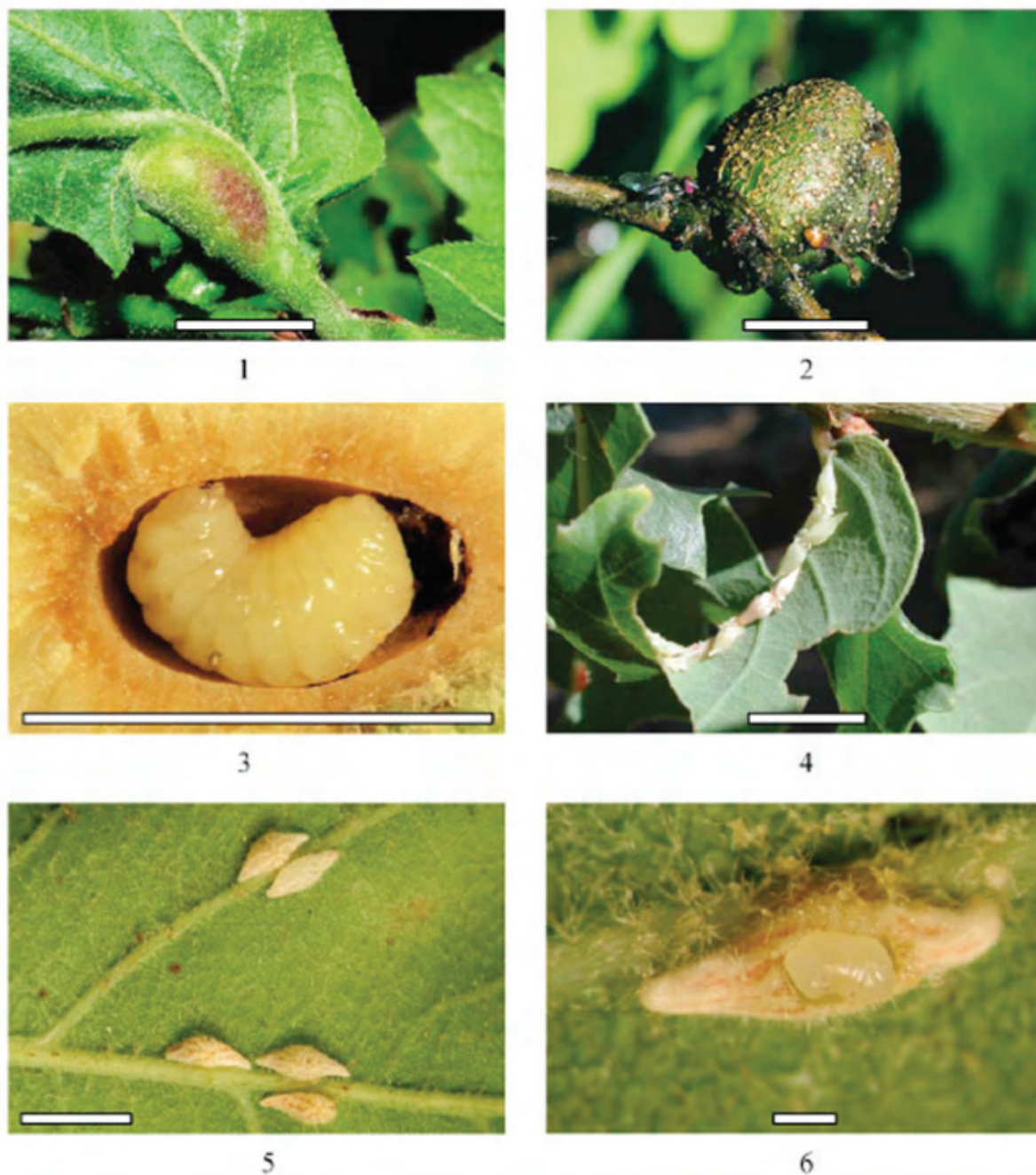


**Plate 28.** 1. *Plagiotrochus gibbosus* sexual gall (scale bar 1 cm) ©JP. 2–3. *Plagiotrochus marianii* asexual gall (scale bar 1 cm) ©BA. 4. *Plagiotrochus panteli* sexual gall (scale bar 1 cm) ©JLNA. 5–6. *Plagiotrochus quercusilicis* sexual galls (scale bars 1 cm, 1mm), in 6. opened to show multiple larval chambers ©JLNA. 7. *Plagiotrochus razeti* asexual gall (scale bar 1 cm) ©JLNA. 8. *Plagiotrochus razeti* sexual gall (scale bar 1mm) ©JLNA.

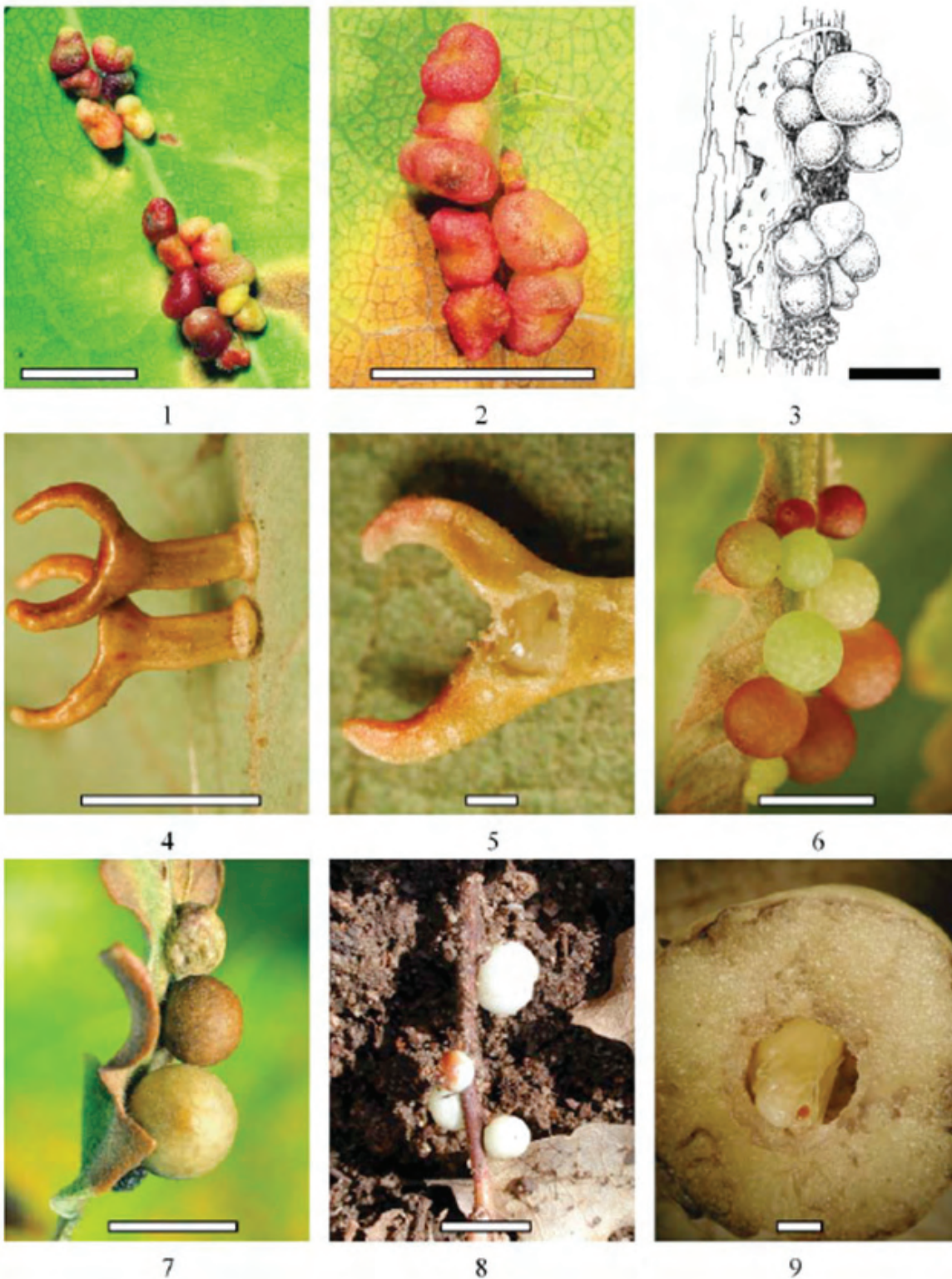


**Plate 29.** 1–2. *Plagiotrochus suberi* asexual galls (scale bars 1 cm) ©JP. 3. *Plagiotrochus vilageliui* (scale bar 1 cm) ©JP. 4. *Plagiotrochus yeusei* asexual gall (scale bar 1 cm) ©JLNA. 5–7. *Pseudoneuroterus macropterus* asexual galls (scale bars 1 cm) ©GS, GyCs, GyCs.





**Plate 30.** 1. Young *Synophrus politus* sexual gall on midrib (scale bar 1 cm) ©GyCs. 2. *Synophrus politus* sexual gall on twig (scale bar 1 cm) ©GyCs. 3. *Synophrus politus* sexual gall dissected with larva (scale bar 1 cm) ©GyCs. 4. *Trigonaspis baeticus* asexual gall (scale bar 1 cm) ©JLNA. 5. *Trigonaspis brunneicornis* asexual gall (scale bar 1 cm) ©JLNA. 6. *Trigonaspis brunneicornis* asexual gall opened to show the larva within (scale bar 1mm) ©JLNA.



**Plate 31.** 1–2. *Trigonaspis megaptera* asexual galls (scale bars 1 cm) ©GyCs. 3. *Trigonaspis megaptera* sexual galls (scale bar 1 cm) ©BA. 4–5. *Trigonaspis mendesi* asexual galls (scale bar 1 cm, 1mm), opened in 5 to show the location of the larva within ©JLNA. 6–7. *Trigonaspis synaspis* asexual galls (scale bars 1 cm) ©JLNA, GyCs. 8–9. *Trigonaspis* sp. (*T. megaptera* or *synaspis*) sexual galls (scale bar 1 cm, 1mm) ©JLNA.



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**Evaluating the use of *Torymus sinensis* against the chestnut gall wasp *Dryocosmus kuriphilus* in the Canton Ticino, Switzerland**

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