Distribution, Hosts and Parasitoids of *Monochamus galloprovincialis* (Coleoptera: Cerambycidae) in Portugal Mainland

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Abstract. The geographic distribution of the pine sawyer (*Monochamus galloprovincialis*), vector of the pine wood nematode (*Bursaphelenchus xylophilus*), is presented for Portugal mainland, according to the UTM 10x10km square grid. Field surveys were conducted in an area of approximately 15% of the maritime pine (*Pinus pinaster*) forested area. Simultaneously, a literature review was made for the previous references on the insect in Portugal. The insect vector was found in 94% of the surveyed locations, suggesting a widespread distribution. A total of eight forest and ornamental conifers were sampled, and for the first time the pine sawyer was detected in Portugal in two of them, namely *Pinus sylvestris* and *Pinus halepensis*. The beetle was not found associated with *Pinus pinea*, *Pinus radiata*, *Cupressus lusitanica*, *Larix decidua* and *Chamaecyparis lawsoniana*. The parasitoid guild associated with the pine sawyer in Portugal was reviewed and updated, with new geographic locations for a total of seven species.

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Key words: Cerambycidae, *Pinus pinaster*; Pine Wood Nematode, *Cyanopterus flavator,* geographic distribution

Distribuição, hospedeiros e parasitóides de *Monochamus galloprovincialis* em Portugal Continental

Sumário. É apresentada e discutida a distribuição geográfica em Portugal Continental do cerambicídeo *Monochamus galloprovincialis*, vetor do nemátode da madeira do pinheiro (*Bursaphelenchus xylophilus*), baseada na grelha UTM 10x10km. Foram realizadas prospeções em aproximadamente 15% da área florestal de pinheiro-bravo (*Pinus pinaster*). Simultaneamente, foi efetuada uma extensa revisão bibliográfica sobre as referências existentes para este cerambicídeo em Portugal. Foi confirmada a sua presença em 94% dos locais estudados, sugerindo uma distribuição alargada e generalizada. Foram prospetadas um total de oito espécies florestais e ornamentais, tendo-se encontrado pela primeira vez em Portugal a associação do inseto vetor com outros hospedeiros, nomeadamente com *Pinus sylvestris* e *Pinus halepensis*. Não se detetou a presença do inseto em *Pinus pinea*, *Pinus radiata*, *Cupressus lusitanica*, *Larix decidua* e *Chamaecyparis lawsoniana*. Adicionalmente, foi também revista e atualizada a comunidade de parasitóides associada a este cerambicídeo, num total de sete espécies, para as quais são apresentadas novas localizações geográficas.

Palavras-chave: Cerambycidae, *Pinus pinaster*, Nemátode da Madeira do Pinheiro, *Cyanopterus flavator,* distribuição geográfica

Distribution des hôtes et parasitoïdes de *Monochamus galloprovincialis* (Coleoptera: Cerambycidae) au Portugal continental

Résumé. La répartition géographique du scieur du pin (*Monochamus galloprovincialis*), vecteur du nématode du bois de pin (*Bursaphelenchus xylophilus*), est présentée au niveau du territoire continental portugais selon le quadrillage UTM 10x10km. Sur le terrain les études ont été menées en 15% environ de la surface forestière de pin maritime (*Pinus pinaster*). Simultanément a été faite une révision bibliographique des références précédentes de l'insecte au Portugal. Sa présence a été confirmée en 94% des endroits étudiés, ce qui suggère une répartition généralisée. Un total de huit conifères forestières et ornementales ont été échantillonnés, et le scieur de pin a été détecté pour la première fois au Portugal sur deux d'entre eux, a savoir *Pinus sylvestris* et *Pinus halepensis*. Le coléoptère n'a pas été trouvé associé à *Pinus pinea, Pinus radiata, Cupressus lusitanica*, *Larix decidua* et *Chamaecyparis lawsoniana*. La guilde des parasitoïdes associés au scieur de pin au Portugal a été revue et mise à jour, avec de nouvelles zones géographiques pour un total de sept espèces.

Mots-clés: Cerambycidae, *Pinus pinaster*, Nématode du Bois de Pin, *Cyanopterus flavator,* répartition géographique

Introduction

One of the most important sanitary problems affecting the maritime pine (*Pinus pinaster* Aiton) is the pine wilt disease (PWD). The causal agent is the pine wood nematode (PWN) *Bursaphelenchus xylophilus* (Steiner and Buhrer) Nickle (Nematoda: Tylenchida: Aphelenchoididae), which was first reported in Portugal by MOTA *et al*. in 1999. To be dispersed the PWN requires a vector insect, being the most effective the beetles of the genus *Monochamus* Dejan (Coleoptera: Cerambycidae) (KOBAYASHI *et al*., 1984; LINIT 1988; KISHI 1995; NAVES *et al*., 2007). In Portugal, the sole vector of the PWN is the pine sawyer *Monochamus galloprovincialis* (Olivier) (SOUSA *et al*., 2001). This beetle was firstly reported in the end of the XIXth century by OLIVEIRA (1894), who collected specimens from the Leiria region which he erroneously identified as *Monochamus sutor* Linnaeus. The species remains absent in subsequent catalogues and collection during the XXth century (CORRÊA DE BARROS, 1916; NEVES, 1950), until it was collected by NEVES *et al*. (1978) in burned *P. pinaster* trees near Arganil, central Portugal.

The pine sawyer is widely distributed in Europe (except in the United Kingdom, Ireland and Cyprus), being common in the Mediterranean area, namely in Italy, France, Spain, and Portugal (FRANCARDI and PENNACCHIO, 1996; VIVES, 2000; BRUSTEL *et al*., 2002; KOUTROUMPA, 2007). Outside of Europe, *M. galloprovincialis* can also be found in North Africa, the Caucasus, Russia, China and Mongolia (HELLRIGL, 1971).

Due to the increasing importance of this species as vector of the PWN, several recent studies have detailed various aspects of its biology and ecology (e.g. KOUTROUMPA *et al*., 2008; NAVES *et al*., 2008; AKBULUT, 2009), the application of chemical substances to control it (SOUSA *et al*., 2013) and its natural enemies (NAVES *et al*., 2005; PETERSEN-SILVA *et al*., 2012). Despite the recently-acquired knowledge, the PWN has continued to spread and is now causing mortality on pine forests in central and northern Portugal (SOUSA *et al*., 2011), (Figure 1). Even though the human transport of infested wood is responsible for long-distance dispersal of the disease, the beetle's flight activity and dispersal is also important when considering the spreading of PWD at a local scale. A detailed knowledge on the distribution and hosts of the vector beetle is important to understand the establishment and incidence of wilt disease in the terrain, and is lacking for Portugal. In this paper we present the first mapping of the distribution and hosts of *M. galloprovincialis* in continental Portugal, and review

the parasitoid guild with new geographic records. A detailed bibliographic review on *M. galloprovincialis* distribution in Portugal was conducted.

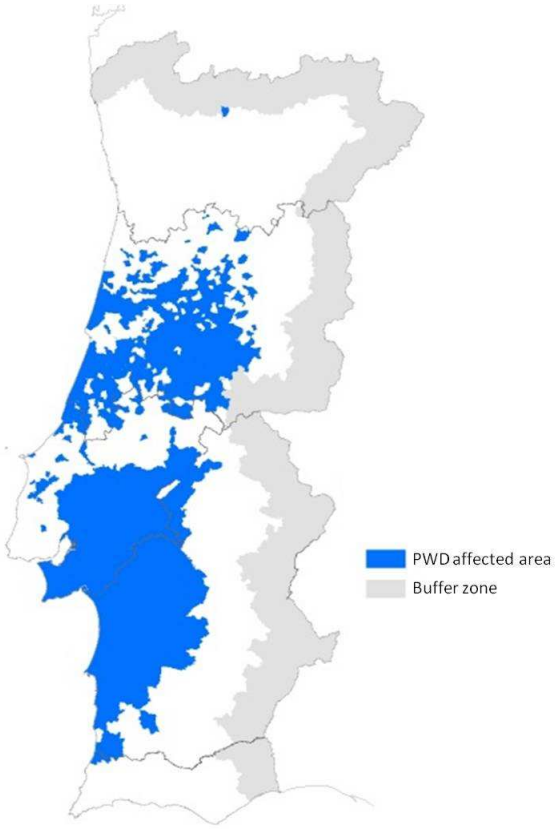

Figure 1 *– Detail of the area currently affected by the pine wilt disease in continental Portugal. Figure adapted from ICNF (2014)*

Materials and methods

Geographic distribution and hosts of Monochamus galloprovincialis

Surveys in the terrain were conducted by the INIAV (Instituto Nacional de Investigação Agrária e Veterinária, Oeiras, Portugal) entomological team. The surveys were mostly directed to areas where *P. pinaster* is the dominant tree

species (ICNF, 2010), recording the locations in the UTM 10x10 km coordinates system (Universal Transverse Mercator coordinate system) and on the NUTS II system (Nomenclature of Territorial Units for Statistics). Surveys braced approximately 15% of the *P. pinaster* forested area. The surveyed areas were chosen based on the current distribution of the zones affected by the pine wilt disease (Figure 1, Annex 1), and on the proximity with Spain (buffer zone). The distribution of *M. galloprovincialis* was assessed by the presence of adult insects or larval instars inside decaying or dead trees. The characteristic round emergence holes made by the adults also allowed identifying past presence of the pine sawyer, but wood dissection was necessary to distinguish from similar holes made by carpenter bees of the genus *Xylocopa* Latreille or by wood wasps of the genus *Sirex* Linnaeus.

Although the majority of the survey was carried on *P. pinaster*, other conifers were also sporadically sampled, namely *Pinus halepensis* Mill., *Pinus pinea* L., *Pinus sylvestris* L., *Pinus radiata* Don, *Cupressus lusitanica* Mill., *Larix decidua* Mill. and *Chamaecyparis lawsoniana* (A. Murraybis) Parl.

Table 1 - *Tree species surveyed for the presence of Monochamus galloprovincialis and parasitoids associated with the species; percentage of total analyzed territory in Portugal mainland according to the NUTS II system (UTM grid of 10 x 10km)*

Tree species surveyed for the presence of *M. galloprovincialis*	**NUTS II**				
	Norte (1)	**Centro (2)**	**Lisboa (3)**	**Alentejo (4)**	**Algarve (5)**
Chamaecyparis lawsoniana	x				
Cupressus lusitanica	x				
Larix decidua	x				
Pinus halepensis			x		
Pinus pinaster	x	x	x	x	x
Pinus pinea			x		x
Pinus radiata	x				
Pinus sylvestris	x				
Percentage of UTM analyzed for parasitoid presence; (Nb)	1% (2)	4% (11)	0% (0)	2% (7)	3.5% (2)
Percentage of UTM analyzed for *M. galloprovincialis* presence (%); (Nb)	8.5% (21)	12.5% (38)	31.5% (11)	5.5% (18)	22% (13)

x- Presence of *M. galloprovincialis* confirmed; Nb- total number of analyzed UTM

Parasitoid guild associated with Monochamus galloprovincialis

The parasitoid guild distribution was studied from 2002 to 2003 and from 2011 to 2014. Locations where the surveys were conducted were randomly chosen among the locations surveyed for *M. galloprovincialis* distribution (Table 1, Annex 2). Field methods were based in the felling of trees with *M. galloprovincialis* presence and the creation of trap trees in the field. Collected wood was transported and reared at the INIAV laboratory, as described in NAVES *el al*. (2005) and PETERSEN-SILVA *et al*. (2012). The identification of parasitoid specimens was confirmed by Dr. Sergey Belokobylskij (Polish Academy of Sciences, Warszawa, Poland) and Dr. Dmitri Kasparyan (St. Petersburg, Russia).

Distribution data

Maps were created with the ESRI® ArcMap™ 10.0 program, and the presence/absence of *M. galloprovincialis* and the parasitoid guild was recorded in the UTM square grid (10 x 10 km) for continental Portugal. New records are signaled in the tables.

Results

Geographic distribution and hosts of Monochamus galloprovincialis

A total of 96 UTM squares with dominant forest cover of *Pinus pinaster* were surveyed over the years (Table 1, Figure 2, and Annex 1). The pine sawyer *M. galloprovincialis* was found in 90 of them (93.8%), being widely distributed in the Portuguese continental territory. This cerambycidae showed an obvious preference for the maritime pine, although it was also found associated with other hosts such as *P. sylvestris* in Viana do Castelo (UTM NG21), and *Pinus halepensis* in Cascais (UTM MC68). All of these are new host records for Portugal. The pine sawyer was not detected in other surveyed conifers, such as *Pinus pinea*, *Cupressus lusitanica*, *Larix decidua* and *Chamaecyparis lawsoniana.*

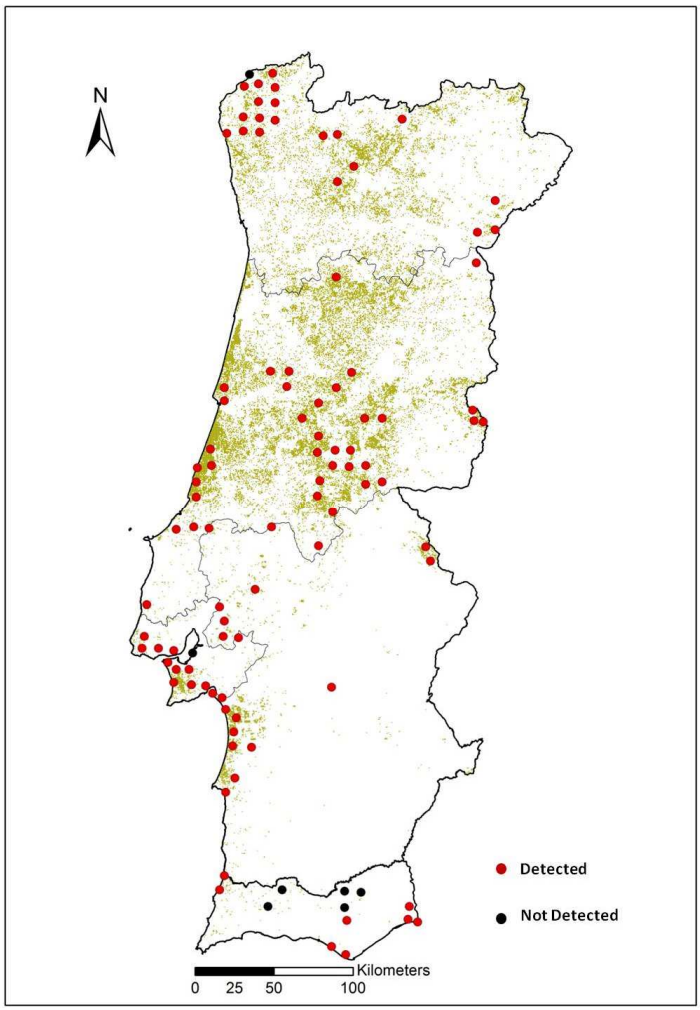

***Figure 2** - Distribution of Monochamus galloprovincialis in continental Portugal (NUTS II grid)*

Parasitoid guild associated with Monochamus galloprovincialis

The surveys lead to the collection of 188 parasitoid specimens found associated with *M. galloprovincialis* larvae. Specimens belong to the families Ichneumonidae and Braconidae and include seven species, namely: *Atanycolus denigrator* (L.); *Atanycolus ivanowi* (Kokujev); *Cyanopterus flavator* (Fabricius);

Coeloides sordidator Ratzeburg; *Doryctes striatellus* (Nees); *Iphiaulax impostor* (Scopoli) and *Xorides depressus* (Holmgren). Their distribution, percentage of species and the UTM grid reference can be found in Table 1, Figure 3, and Annex 2, of which most are new distribution localities for Portugal. *Cyanopterus flavator* is the more frequently detected species, being found in seven of the 15 locations analyzed.

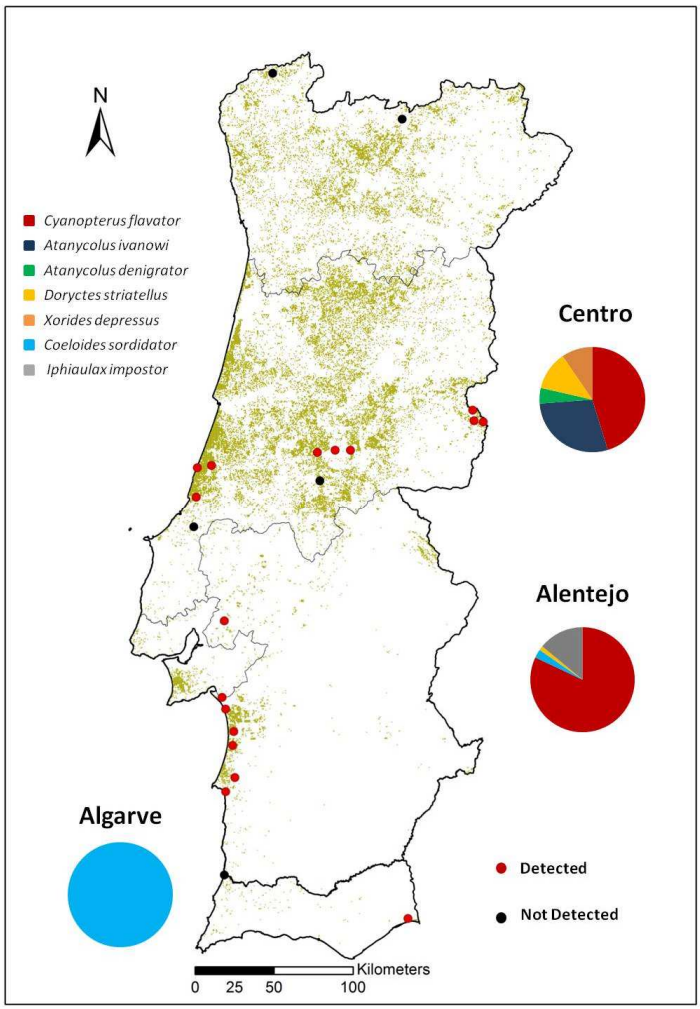

Figure 3 *- Distribution (NUTS II grid) and percentage of parasitoid species associated with Monochamus galloprovincialis in continental Portugal*

Conclusions/ Discussion

The pine sawyer was found to be widely distributed on most of the Portuguese territory where pine forests are abundant, not being conditioned by edapho-climatic variations of the different localities, which was to be expected considering its vast distribution range (HELLRIGL, 1971). The absence of this widespread and conspicuous species from the previous catalogues of OLIVEIRA (1894), CORRÊA DE BARROS (1916), NEVES (1950) and SERRANO (1983) is somewhat intriguing, although these authors were not specifically looking for this beetle, unlike the surveys conducted by the INIAV team. It is also possible that the pine sawyer may have benefited from the great campaigns of forestation with *P. pinaster* that occurred in Portugal during the XXth century, experiencing a distribution expansion due to the higher availability of hosts. Insect populations may have also benefited from the sanitary problems and forest fires which occurred after this forest expansion, as the dead and scorcher pines tend to attract *Monochamus* beetles for breeding (BYERS, 1989a; b; SATO and MAETO, 2006; BONIFÁCIO *et al*., 2012).

Although maritime pine is clearly the preferred host in Portugal, the association of *M. galloprovincialis* with other hosts such as *P. sylvestris* and *P. halepensis*is is not surprising, as these are very frequent hosts all over the Mediterranean basin (HELLRIGL, 1971; EVANS *et al*., 1996; FRANCARDI and PENNACCHIO, 1996).Other unusual hosts include *Pinus strobus* (VINCENT, 2007), *Pinus uncinata* (BEHALOVÁ, 2006) and even *Picea abies* (L.) H. Karst. and *Picea orientalis* (L.) Peterm. (YUKSEL, 1998), although all of these species are inexistent or rare in Portugal.

Regarding the parasitoids associated with the insect vector, a fairly diverse guild was detected over the years, being dominated by *Cyanopterus flavator*, the most abundant species and with the widest distribution. Nevertheless and as already mentioned by NAVES *et al*. (2005) and PETERSEN-SILVA *et al*. (2012), these are all generalist species which attack a vast array of Cerambycidae and even other coleopteran, and therefore, their use as biological control agents seems ineffective and unlikely. Future samplings should focus on other natural enemies, such as bacteria or fungi, which have been found to affect other *Monochamus* species elsewhere, and have not yet been studied in detail in Portugal.

This is a first approach to elaborate a detailed mapping on the distribution range and hosts of *M. galloprovincialis* in Portugal, and may constitute an important tool for future assessments of pine wilt disease distribution and

dispersal, and for the implementation of management and control strategies of this complex forest sanitary problem.

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Annexes

Annex 1

Distribution of *Monochamus galloprovincialis* in continental Portugal (UTM grid of 10 x 10km) and hosts

(a) – New host for *M. galloprovincialis* in Portugal; 1- Ribeiro, 1992; 2- Neves, *et al*., 1978; 3- Oliveira, 1894; Unknown – *M. galloprovincialis* adult specimens collected outside the host.

Parish	**UTM reference**	**Host**	***Monochamus galloprovincialis***
Abrantes (2)	ND75	*P. pinaster*	Detected
Alcácer do Sal (4)	NC24	*P. pinaster*	Detected
Alcobaça (2)	MD99	*P. pinaster*	Detected
	ND06	*P. pinaster*	Detected
Aljezur (5)	NB13	*P. pinaster*	Detected
Arcos de Valdevez (1)	NG43	*P. pinaster*	Detected
	NG44	*P. pinaster*	Detected
Arganil[2] (2)	NE85	*P. pinaster*	Detected
Barreiro (3)	MC97	*P. pinaster*	Detected
Benavente (4)	NC19	*P. pinaster*	Detected
	ND10	*P. pinaster*	Detected
	ND11	Unknown	Detected
Caldas da Rainha (2)	MD86	*P. pinaster*	Detected
	MD96	*P.pinaster*	Detected
Cascais (3)	MC68	*P. halepensis*	Detected[(a)]
Castelo Branco (2)	PE13	*P. pinaster*	Detected
Castro Daire (2)	NF82	*P. pinaster*	Detected
Castro Marim (5)	PB31	*P. pinaster*	Detected
	PB32	*P. pinaster*	Detected
	PB41	*P. pinaster*	Detected
Celorico de Basto (1)	NF88	*P. pinaster*	Detected
Chaves (1)	PG22	*P. pinaster*	Detected
Évora[1] (4)	NC86	*P. pinaster*	Detected
Faro (5)	NA89	*P. pinaster*	Detected
		P. pinea	Not detected
	NA99	*P. pinaster*	Detected
Figueira da Foz (2)	NE14	*P. pinaster*	Detected
	NE15	*P. pinaster*	Detected
Figueira de Castelo Rodrigo (2)	PF73	*P.pinaster*	Detected
Figueiró dos Vinhos (2)	NE63	*P. pinaster*	Detected
Freixo de Espada a Cinta (1)	PF85	*P. pinaster*	Detected
Fundão (2)	PE03	*P. pinaster*	Detected
Góis (2)	NE74	*P. pinaster*	Detected
Golegã (4)	ND46	*P. pinaster*	Detected

Grândola (4)	NC14	*P. pinaster*	Detected
	NC15	*P. pinaster*	Detected
	NC22	*P. pinaster*	Detected
	NC23	*P. pinaster*	Detected
	NC32	*P. pinaster*	Detected
Idanha-a-Nova (2)	PE73	*P. pinaster*	Detected
	PE83	*P. pinaster*	Detected
Leiria[3] (2)	NE01	*P. pinaster*	Detected
Lisboa (3)	MC88	Unknown	Detected
Loulé (5)	NB92	*P. pinaster*	Not detected
	NB93	*P. pinaster*	Not detected
Mação (2)	ND87	*P.pinaster*	Detected
Mafra (3)	MD61	*P. pinaster*	Detected
Marinha Grande (2)	ME90	*P. pinaster*	Detected
	NE00	*P. pinaster*	Detected
Mealhada (2)	NE46	*P. pinaster*	Detected
Mogadouro (1)	PF87	*P. pinaster*	Detected
Monção (1)	NG45	*P. pinaster*	Detected
Monchique (5)	NB42	*P. pinaster*	Not detected
Montalegre (1)	NG81	*P. pinaster*	Detected
Montijo (3)	MC98	*P. pinea*	Not detected
	NC29	*P. pinaster*	Detected
Nazaré (2)	MD98	*P. pinaster*	Detected
Odemira (4)	NB14	*P. pinaster*	Detected
Oeiras (3)	MC78	*P. pinaster*	Detected
Oleiros (2)	NE91	*P. pinaster*	Detected
Oliveira do Hospital (2)	NE96	*P. pinaster*	Detected
Paredes de Coura (1)	NG33	*P. pinaster*	Detected
	NG34	*P. pinaster*	Detected
		P. sylvestris	Not detected
Pedrógão Grande (2)	NE72	*P. pinaster*	Detected
Penacova (2)	NE55	*P. pinaster*	Detected
	NE56	*P. pinaster*	Detected
Penamacor (2)	PE74	*P. pinaster*	Detected
Ponte da Barca (1)	NG42	*P. pinaster*	Detected
Ponte de Lima (1)	NG31	*P. pinaster*	Detected
		P. sylvestris	Not detected
	NG32	*P. pinaster*	Detected
Portalegre (4)	PD44	*P. pinaster*	Detected
	PD45	*P. pinaster*	Detected
Proença-a-Nova (2)	NE90	*P. pinaster*	Detected
	PE00	*P. pinaster*	Detected
Ribeira de Pena (1)	NF99	*P. pinaster*	Detected
Salvaterra de Magos (4)	ND32	*P. pinaster*	Detected
Santiago do Cacem (4)	NC20	*P. pinaster*	Detected
São Brás de Alportel (5)	NB91	*P. pinaster*	Detected
Sardoal (2)	ND78	*P. pinaster*	Detected
Seixal (3)	MC77	*P. pinaster*	Detected
	MC87	*P. pinaster*	Detected
Sertã (2)	NE71	*P. pinaster*	Detected
	NE80	*P. pinaster*	Detected
	NE81	*P. pinaster*	Detected

Sesimbra (3)	MC86	*P. pinaster*	Detected
	MC96	*P. pinaster*	Detected
	NC05	*P. pinaster*	Detected
		P. pinea	Not detected
Setúbal (3)	NC06	*P. pinaster*	Detected
Silves (5)	NB53	*P. pinaster*	Not detected
Sines (4)	NB19	*P. pinaster*	Detected
		P. pinaster	Detected
Sintra (3)	MC69	*P. pinaster*	Detected
Tavira (5)	PB03	*P. pinaster*	Not detected
Torre de Moncorvo (1)	PF75	*P. pinaster*	Detected
Valença (1)	NG25	*P. radiata*	Not detected
Viana do Castelo (1)	NG11	*P. pinaster*	Detected
	NG21	*P. sylvestris*	Detected[(a)]
	NG22	*P. pinaster*	Detected
Vieira do Minho (1)	NG71	*P. pinaster*	Detected
		P. sylvestris	Not detected
		C. lusitanica	Not detected
		L. decidua	Not detected
		C. lawsoniana	Not detected
Vila de Rei (2)	ND79	*P. pinaster*	Detected
Vila Nova de Cerveira (1)	NG24	*P. pinaster*	Detected
		P. radiata	Not detected
Vila Velha de Rodão (2)	PD09	*P. pinaster*	Detected
	PD19	*P. pinaster*	Detected

(a) – New *Monochamus galloprovincialis* host for the Portuguese territory; (Nb) – Number of the NUTS II region associated to each parish. 1-Norte, 2-Centro, 3-Lisboa, 4-Alentejo, 5-Algarve

Annex 2

Distribution (UTM grid of 10 x 10km) of parasitoid species associated with *Monochamus galloprovincialis* in continental Portugal

Parish (NUTS II)	UTM reference	Parasitoid presence (Nb of specimens)
Benavente (4)	ND10	*C. flavator* (6)
Caldas da Rainha (2)	MD96	Not detected
Castro Marim (5)	PB31	*C. sordidator*[(a)] (1)
Chaves (1)	PG22	Not detected
Grândola (4)	NC14 NC15 NC22 NC23	*C. flavator* (107) ; *C. sordidator*(5); *I. impostor* (21)
Idanha-a-Nova (2)	PE83 PE73	*A. ivanowi* (6); *C. flavator* (7)
Leiria (2)	MD98	*D. striatellus*[(a)] (1)
Marinha Grande (2)	NE00 ME90	*A. denigrator* (2); *C. flavator* (7); *D. striatellus* (2); *X. depressus* (3)
Monção (1)	NG45	Not detected
Odemira (4)	NB14	Not detected
Oleiros (2)	NE91	*D. striatellus*[(a)] (1); *C. flavator*[(a)] (2); *X. depressus*[(a)] (1)
Penamacor (2)	PE74	*A. ivanowi* (6); *C. flavator* (7)
Santiago do Cacém (4)	NC20	*D. striatellus*[(a)] (1)
Sertã (2)	NE71 NE81	*C. flavator*[(a)] (3); *D. striatellus*[(a)] (1)
Sines (4)	NB19	*D. striatellus*[(a)] (1)
Vila de Rei (2)	ND79	Not detected

(a) – New geographical location in the Portuguese territory; (Nb) – Number of the NUTS II region associated to each parish. 1-Norte, 2-Centro, 3-Lisboa, 4-Alentejo, 5-Algarve