# THE CRAYFISH PLAGUE FUNGUS (*APHANOMYCES ASTACI*) IN SPAIN.

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## ABSTRACT

Since 1978 there has been a series of outbreaks of crayfish plague fungus, *Aphanomyces astaci* in Spain. As a result, most populations of the native freshwater crayfish species, *Austropotamobius pallipes*, have been devastated by this disease. This paper describes the steady spread of the crayfish plague through populations of *A. pallipes* since 1978 and the present situation of the crayfish plague. Thus, the first diagnosis of this disease since 1978 and the first isolation of *A. astaci* from *A. pallipes* in Spain are also presented.

**Key-words :** *Oomycetes*, *Aphanomyces*, *Saprolegnia*, crayfish, epidemiology.

## INFECTION FONGIQUE À *APHANOMYCES ASTACI* OU «PESTE» DES ÉCREVISSES EN ESPAGNE.

## RÉSUMÉ

Depuis 1978, une série d'épizooties correspondant à la «peste» des écrevisses, dont l'agent responsable est le champignon *Aphanomyces astaci*, s'est produite en Espagne. En conséquence, la plupart des populations de l´écrevisse patrimoniale *Austropotamobius pallipes* a été décimée par cette maladie. Cet article décrit l'état actuel de la maladie dans les populations d'*A. pallipes* depuis 1978 et la situation actuelle qui en résulte. De plus, le premier diagnostic de la maladie est décrit depuis 1978 et le premier isolement du champignon est réalisé à partir d'un stock d'*A. pallipes* infectées et mortes sur le territoire espagnol.

**Mot-clés :** *Oomycetes*, *Aphanomyces*, *Saprolegnia*, écrevisse, épidémiologie.

## INTRODUCTION

The animal pathogenic fungus *Aphanomyces astaci (Oomycetes)* is responsible for the crayfish plague, a disease which has devastated many European native populations of freshwater crayfish (UNESTAM, 1973 ; ALDERMAN *et al.*, 1984 ; SMITH and SÖDERHÄLL 1986 ; TAUGBØL *et al.*, 1993).

*A. astaci* is a parasitic fungus specialized in living in the cuticle of freshwater crayfish and no natural host other than crayfish is known (UNESTAM, 1969 a, b). European, Japanese and Australian freshwater crayfish species have been found to be highly susceptible to the crayfish plague fungus (UNESTAM, 1969 a). Mortalities occur in percentages of 100 % for all these highly susceptible species after fungal attack (UNESTAM, 1969 a ; ALDERMAN and POLGLASE, 1988). The mycelia grow rapidly through the cuticle, and reach the internal body cavity, which results in crayfish death within 6-10 days (UNESTAM and WEISS, 1970 ; UNESTAM, 1972). However, in the North American species of crayfish, such as signal crayfish, *Pacifastacus leniusculus,* spiny-cheek crayfish *Orconectes limosus* and red-swamp crayfish, *Procambarus clarkii,* the fungus elicits strong defence reactions immediately upon penetration of the cuticle and as a consequence melanin is deposited on the hyphae (PERSSON *et al.*, 1987 ; VEY *et al.*, 1983 ; DIÉGUEZ-URIBEONDO and SÖDERHÄLL, 1993, respectively). It appears that this disease is endemic of North America and that the higher resistance of North American species is a result of a coevolution of fungus and host (UNESTAM, 1969 a ; UNESTAM and WEISS, 1970 ; UNESTAM, 1972). Thus, North American species are carrying this fungus as a chronic infection in melanized spots in their cuticle, named «black spots» (VEY *et al.*, 1983 ; PERSSON and SÖDERHÄLL, 1983 ; PERSSON *et al.*, 1987 ; DIÉGUEZ-URIBEONDO and SÖDERHÄLL, 1993).

In Spain, it is known that at least two North American crayfish species were introduced during the 1970's, *Pr. clarkii* in the southern parts of Spain (Badajoz in 1973 and Las Marismas del Guadalquivir near Sevilla in 1974) and *P. leniusculus* in the north central areas of Spain (Guadalajara in 1974, Soria in 1975, Cuenca and Burgos in 1978) (HABSBURGO-LORENA, 1978 ; BLANCO and CUELLAR, 1982) (Figure 5).

There are not clear data about the first outbreak of this disease in Spain. It is known that sporadic mass mortalities occurred in 1958, 1965 and 1975 (CUELLAR and COLL, 1983) and although it has been speculated that these mortalities could have been due to crayfish plague attacks, no identification of the causal agent was achieved (CUELLAR and COLL, 1983). In 1978, mortalities were reported in river Riaza (Burgos) and river Guadiana (Ciudad Real) and the causative agent was then identified as the crayfish plague fungus, *A. astaci,* by T. UNESTAM (CUELLAR and COLL, 1983). In 1982, most regions of Spain were affected by this epizooty and the distribution of *A. pallipes* was dramatically reduced. As a result, the Spanish native crayfish is currently considered an endangered species and its populations can be found in isolated areas like headwaters and closed waterbodies.

Unfortunately, the status of crayfish plague in Spain after the big hit of the plague (1978-1982) has not been studied. Moreover, there has not been any diagnosis of this disease since this period and in many cases the disappearance of native crayfish has been blamed on crayfish plague without any identification of the causative agent. Therefore, the impact of crayfish plague over native populations remained to be determined.

In this paper, a number of mass mortalities of crayfish has been studied and their causes investigated. Furthermore, the presence of *A. astaci* in several populations of *P. leniusculus* and *Pr. clarkii* has also been studied. Thus, the present situation of crayfish plague in Spain is discussed.

## MATERIAL AND METHODS

### Presence of *A. astaci* in North American species of crayfish

The presence of *A. astaci* in North American species thriving in Spain was investigated. For this purpose, the most representative populations of these two species were studied in some areas of the provinces of Alava, Burgos, Navarra and Guipúzcoa (Table I). Samples of 50 animals at intermoult stage were collected and examinations were done on living crayfish when possible. If not, samples were preserved in ethanol 70 %.

In order to determine the presence of crayfish plague in these exotic species, the crayfish were examined for occurrence of «black spots», *i.e.*, melanized areas, in their cuticle (SÖDERHÄLL *et al.*, 1981), and for each population the number of «black spots» per crayfish was calculated. Pieces of melanized areas or spots were cut out from the cuticle, washed carefully first with ethanol and then several times with sterile water, and examined under microscope. When hyphal growth was observed a piece of the infected area was allowed to grow in peptone glucose medium (PG-1) (SÖDERHÄLL and CERENIUS, 1987) and to sporulate by immersing the growing hyphae in sterile mineral water. When typical *sporangia* of *Aphanomyces* were observed the fungal infection was assessed as to a very likely *A. astaci* infection.

**Table I**

**Some cases of mass mortalities of crayfish in Spain.**

**Tableau I**

**Quelques cas de mortalité massive chez les écrevisses en Espagne.**

| | Year | Species | Origin (basin) | Region | Diagnosis |
|---|---|---|---|---|---|
| 1 | 1997 | *A. pallipes* | Pastrina (Nervión) | Burgos | crayfish plague |
| 2 | 1997 | *A. pallipes* | Tributary of river Bayas (Ebro) | Alava | crayfish plague |
| 3 | 1996 | *A. pallipes* | Pond of Argomaniz (Ebro) | Alava | crayfish plague |
| 4 | 1997 | *A. pallipes* | Arriola (Ebro) | Alava | crayfish plague |
| 5 | 1996 | *A. pallipes* | Tributary of river Arga (Ebro) | Navarra | pollution |
| 6 | 1997 | *A. pallipes* | Val de Murcia (Ebro) | La Rioja | unknown* |
| 7 | 1997 | *A. pallipes* | Gumiel (Duero) | Burgos | crayfish plague |
| 8 | 1995 | *A. pallipes* | Duero river Basin | Burgos | unknown* |
| 9 | 1997 | *A. pallipes* | Fuentenebro (Duero) | Burgos | crayfish plague |
| 10 | 1994 | *A. pallipes* | River Vozmediano (Ebro) | Soria | crayfish plague |
| 11 | 1997 | *A. pallipes* | Royuela (Turia) | Teruel | crayfish plague |
| 12 | 1997 | *A. pallipes* | Tragacete (Júcar) | Cuenca | crayfish plague |
| 13 | 1997 | *A. pallipes* | Arroyo Ermita (Guadalquivir) | Granada | saprolegniasis |
| 14 | 1996 | *A. pallipes* | Crayfish farm | Huesca | unknown* |
| 15 | 1991 | *A. pallipes* | Crayfish farm | Zaragoza | crayfish plague |
| 16 | 1996-97 | *A. pallipes* | Crayfish farm | Ciudad Real | water quality |
| 17 | 1997 | *P. leniusculus* | - | Guipúzcoa | crayfish plague |
| 18 | 1994 | *P. leniusculus* | River Mediano (Ebro) | Navarra | crayfish plague |
| 19 | 1997 | *P. leniusculus* | River Bayas (Ebro) | Alava | unknown* |
| 20 | 1997 | *P. leniusculus* | River Zadorra (Ebro) | Alava | pollution |
| 21 | 1997 | *P. leniusculus* | River Omecillo (Ebro) | Alava | pollution |

* Analyses revealed no fungal infections or other reasons for the mortality.

### Determination of mass mortalities

Samples from 21 different mass mortalities occurring both in native and signal crayfish during the 1990's were investigated (Table I). Mass mortalities of native crayfish originated from wild populations, ca 13, and crayfish farms, ca 3. In addition, five mass mortalities occurring in wild *P. leniusculus* populations were also studied.

Moribund dead crayfish from mass mortalities were brought to the laboratory either fixed in 70 % ethanol or fresh cooled. Diagnosis was performed by following protocol described in CERENIUS *et al.* (1987).

### Isolation and culture of *A. astaci*

Isolation of an *A. astaci* strain was done by following the methods described in CERENIUS *et al.* (1987). Briefly, an infected area was cut out from the cuticle, washed carefully with sterile water and them placed in PG-1. A sterile glass ring was placed around the inoculum to force hyphae emerging from the piece of the cuticle to grow within the agar. In order to deter bacterial growth, 0.5 ml of 0.05 % potassium tellurite was also added to the inoculum inside the glass ring.

The isolated strain was maintained on peptone glucose agar and PG-1 drops cultures. Observations and identifications were made on PG-1 agar and PG-1 drops cultures, using a inverted microscope (200 X and 400 X). Cultures were examined for morphology mode of sporulation and oogonia. Corn meal agar (CMA) was used for searching sexual structures.

## RESULTS

The results showed that all populations studied of *P. leniusculus* and *Pr. clarkii* have «black spots» in their cuticles (Figure 1). The number of «black spots» per crayfish varied among crayfish populations, *i.e.*, 0.01 to 0.2 «black spots» per crayfish in *P. leniusculus* and 0.01 to 0.05 «black spots» per crayfish in *Pr. clarkii* (data not shown).

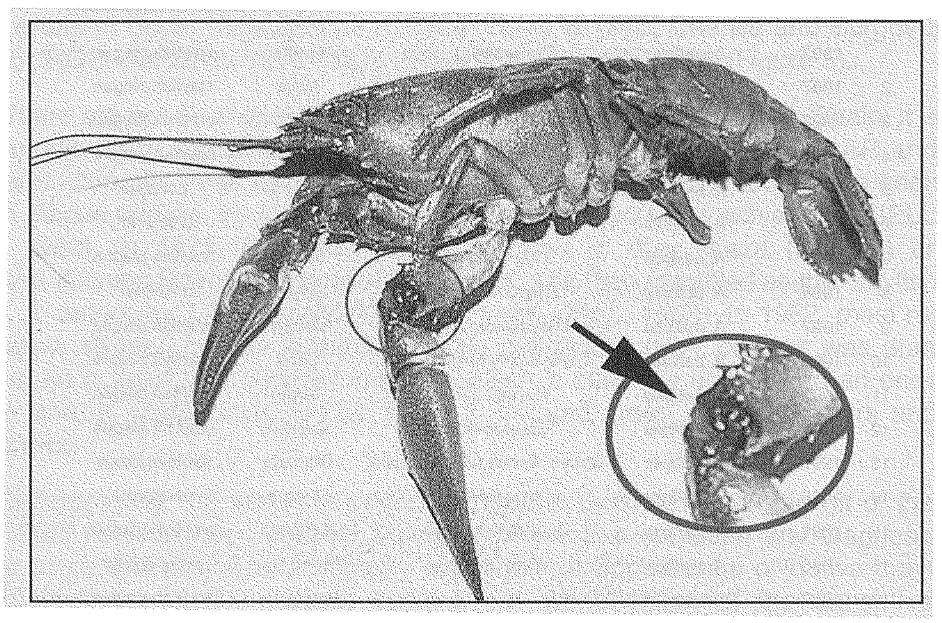

**Figure 1**

**Signal crayfish, *Pacifastacus leniusculus*, from river Arakil (Navarra) with melanized spots (arrows) harbouring the crayfish plague fungus, *Aphanomyces astaci*.**

**Figure 1**

**Ecrevisse signal, *Pacifastacus leniusculus*, provenant de la rivière Arakil (Navarre), et présentant des tâches de mélanine qui sont dues à la «peste» des écrevisses, *Aphanomyces astaci*.**

Microscopical examination of the spots revealed fungal attack in the majority of them (Figure 2). The morphology of the observed hyphae and *sporangia* was typical of *Aphanomyces* spp (Figure 3). Therefore, the spots were very likely caused by the crayfish plague fungus. This idea is supported by the finding that the majority of «black spots» observed both in populations of *P. leniusculus* in Sweden and Finland (SÖDERHÄLL *et al.*, 1981 ; NYLUND and WESTMAN, 1983) and in some specimens of *Pr. clarkii* of Spanish origin (DIÉGUEZ-URIBEONDO and SÖDERHÄLL, 1993) are caused by *A. astaci*.

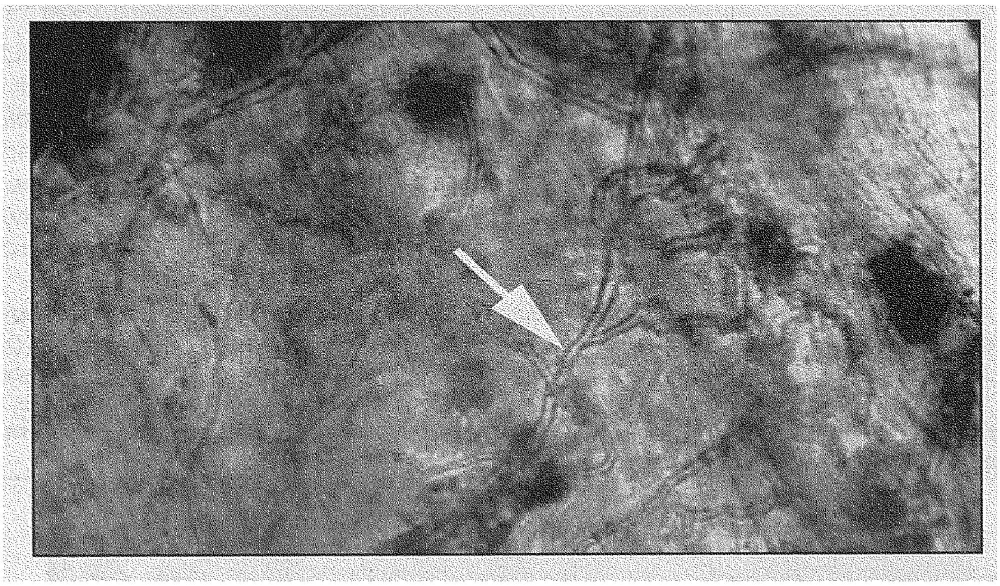

**Figure 2**

**Melanized hyphae of the crayfish plague fungus, *Aphanomyces astaci* (arrow), from a «black spot».**

**Figure 2**

**Hyphae mélanisée du champignon de la «peste» des écrevisses, *Aphanomyces astaci* (flèche), provenant d'une «tâche noire».**

On the other hand, the results obtained from analyses of mass mortalities indicated that 10 out of 16 mortalities in *A. pallipes* were due to crayfish plague (Table I) since microscopical observations of the infective tissues revealed the presence of a typical growth and morphology of this fungus within the cuticle (Figure 4). In wild populations 9 out of 13 mortalities could be assigned to the crayfish plague fungus. In crayfish farm mortalities one case out of three was caused by crayfish plague. In other cases, mortalities were due to other factors such as diseases, *i.e.*, saprolegniasis, different kinds of pollution, *i.e.*, hydrocarbons and pesticides, or the reasons remained unknown. In addition, some outbreaks in wild populations of *P. leniusculus* were detected and the reasons of their disappearance were found to be similar to those encountered in mortalities of *A. pallipes* (Table I).

In most cases fungal isolation could not be achieved because the samples were fixed in ethanol. However, in three cases fresh material was obtained and isolations attempts were made. From the infected tissues of samples of *A. pallipes* from river Pastrina (Burgos) and from a tributary of river Bayas (Alava) typical saprolegniaceous fungus were isolated. These fungus could be assigned to the genus *Aphanomyces* according to their morphology and typical asexual sporulating hyphae (Figures 3 and 4). These isolates were never seen to produce oogonia in corn meal agar, and consequently to determine the species, reinfection experiments were carried out. The fact that these new isolates showed to be virulent towards crayfish prompted us to assign these isolates to the species *A. astaci*.

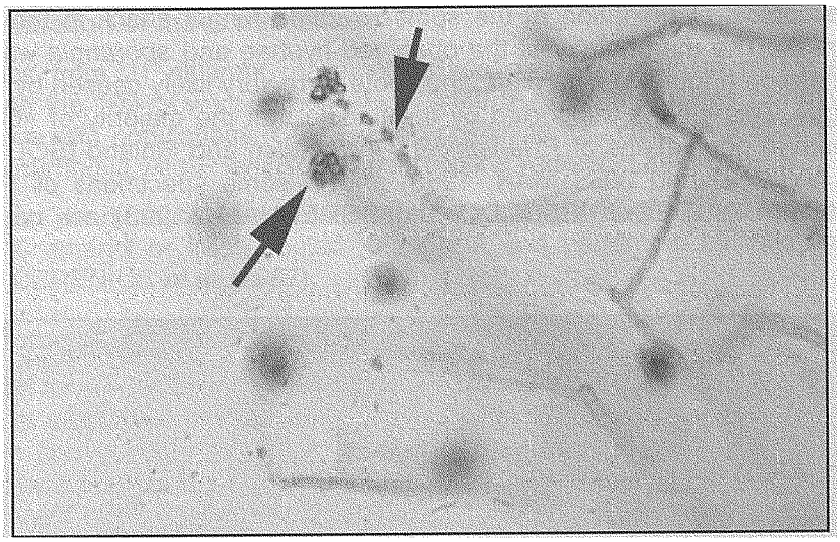

**Figure 3**

**Cuticle of a native crayfish, *Austropotamobius pallipes*, infected with the crayfish plague fungus, *Aphanomyces astaci*.**

**Figure 3**

**Cuticule d'une écrevisse patrimoniale, *Austropotamobius pallipes*, infectée par le champignon de la «peste» des écrevisses, *Aphanomyces astaci*.**

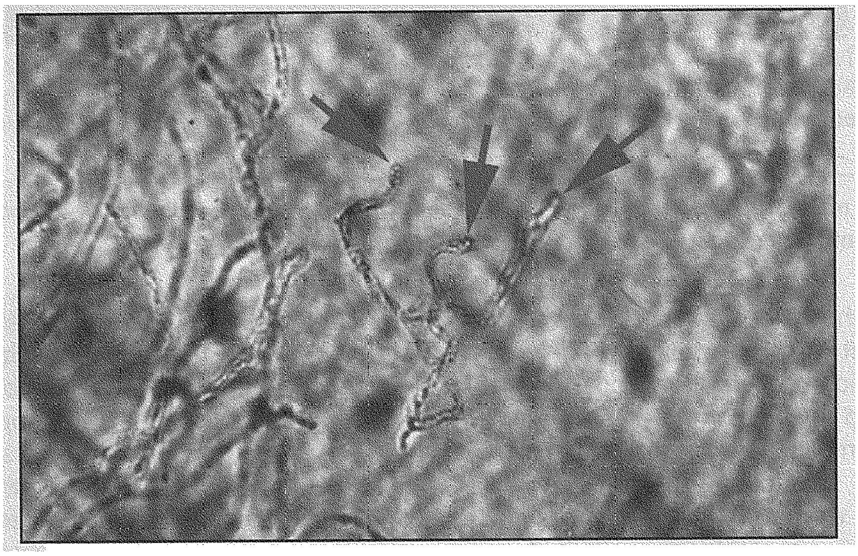

**Figure 4**

**Sporulating hyphae of the crayfish plague fungus, *Aphanomyces astaci*. Arrows show typical *sporangium* of *Aphanomyces* spp, spore balls, and a row of primary spores within the hyphae.**

**Figure 4**

**Sporulation des hyphae du champignon de la «peste» des écrevisses, *Aphanomyces astaci*. Les flèches indiquent les *sporangium* typiques d'*Aphanomyces* spp, les accumulations des spores et une rangée de spores primaires dans l'hyphae.**

In addition, dead crayfish from a tributary of river Guadalquivir in Granada did not show any sign of crayfish plague but tissues were infected by *Saprolegnia* sp. The fungus was certainly infecting the cuticle since a strong melanization response of the crayfish immune system was observed. This fungus was also isolated and is currently being characterized.

## DISCUSSION

The epizootology of the crayfish plague in Spain was first reviewed by CUELLAR and COLL (1983). In this work the year 1958 was mentioned as the possible date of first observation of the crayfish plague in Spain. However, the lack of any identification of the causative agent of the mortalities monitored in 1958, 1965 and 1975, and also the abundance of populations of *A. pallipes* until late 1970's, argue strongly against the idea of the presence of *A. astaci* before the first diagnosis made in river Riaza and river Guadiana in 1978 (Figure 5). In this paper we propose this year, 1978, as the date when the first crayfish plague outbreaks took place in Spain.

CUELLAR and COLL (1983) speculated about a possible relationship between the mortality in river Riaza and the presence of an illegal farm of *Astacus leptodactylus* in the vicinity. Although this possibility cannot be excluded, it is interesting to see that a temporal and a geographical relationship could be established between the first introductions of the red-swamp crayfish and the signal crayfish and the commencement of the epizootic events (Figure 5). It is known that outbreaks of crayfish plague in Europe are in many cases likely to be due to introductions of North American species of crayfish (ALDERMAN and POLGLASE, 1988). Therefore, it is also likely that crayfish plague outbreaks in Spain might have been due to introductions of *Pr. clarkii* and *P. leniusculus*. The presence of this fungus in *Pr. clarkii* of Spanish origin (DIÉGUEZ-URIBEONDO and SÖDERHÄLL, 1993 ; DIÉGUEZ-URIBEONDO *et al.*, 1995) and the finding that wild populations of *Pr. clarkii* and *P. leniusculus* from different areas of Spain are carrying the crayfish plague fungus suggest this idea.

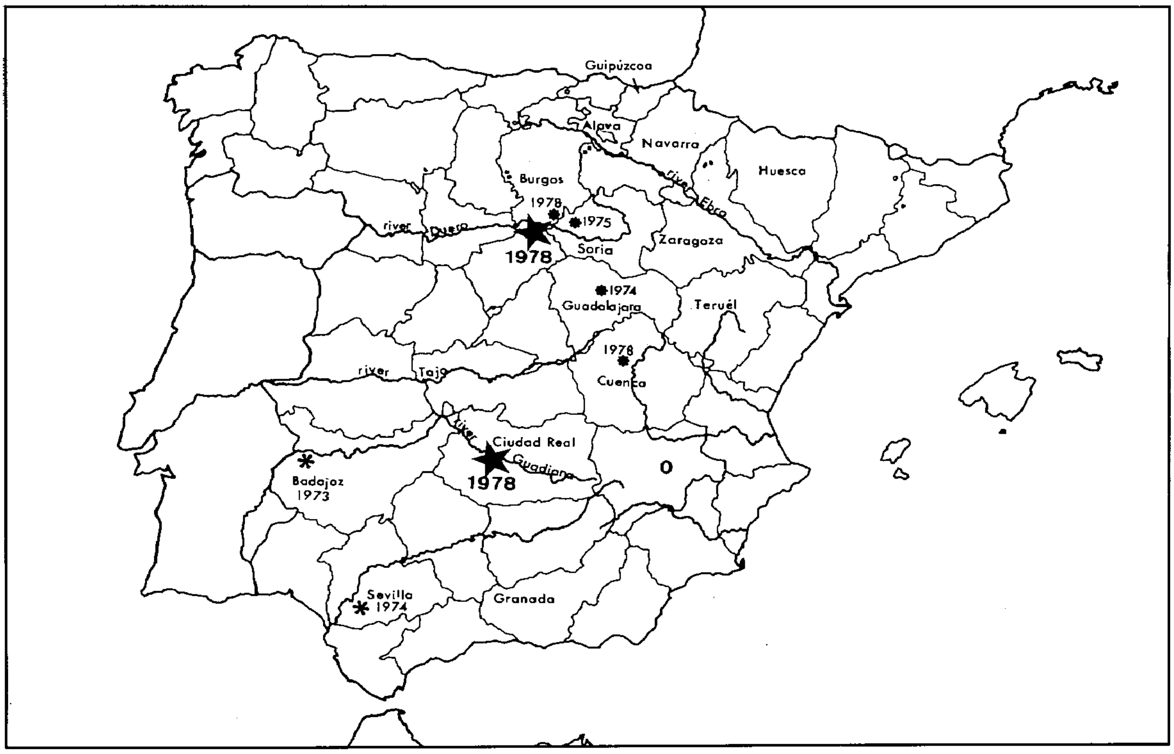


**Figure 5**

**First introductions of North American species of crayfish, *Procambarus clarkii* (*) and *Pacifastacus leniusculus* (✱) in Spain, and the first crayfish plague outbreaks diagnosed in Spain (★).**

**Figure 5**

**Premières introductions des espèces d'écrevisses nord-américaines, *Procambarus clarkii* (*) et *Pacifastacus leniusculus* (✱), et les premiers cas de la «peste» des écrevisses diagnostiqués (★) en Espagne.**

The initial steps of the epizooty were certainly favoured by the abundance of native crayfish and also the active fishery and commerce of crayfish during the 1970's. Thus, the *A. pallipes* populations remained in habitats like headwaters, closed water bodies, brooks, *etc.*, far from the threat of the crayfish plague. Within this context, the present status of the crayfish plague fungus was studied. The results presented in this paper show that the crayfish plague fungus is nowadays still causing mortalities in native crayfish, especially in wild populations (Table I and Figure 6). The establishment of North American crayfish populations and their spread have given rise to «chronic areas of pest» which are very likely responsible for the steady spread of the disease. Interestingly, the crayfish plague fungus has been isolated from mass mortalities in river Pastrina (Burgos) and Bayas basin (Alava). These strains constitute the first isolations of *A. astaci* from *A. pallipes* mortalities in Spain. A recent characterization of these strains by RAPD-PCR demonstrates that they are of signal crayfish origin (DIÉGUEZ-URIBEONDO and SÖDERHÄLL, unpublished). RAPD-PCR has already been shown to be useful in tracking the dissemination of particular strains of *A. astaci* (HUANG *et al.*, 1994). For example, more recent outbreaks of crayfish plague in Sweden and Germany have been shown to be due to introductions of *P. leniusculus* (HUANG *et al.*, 1994 ; OIDTMANN *et al.*, 1997).

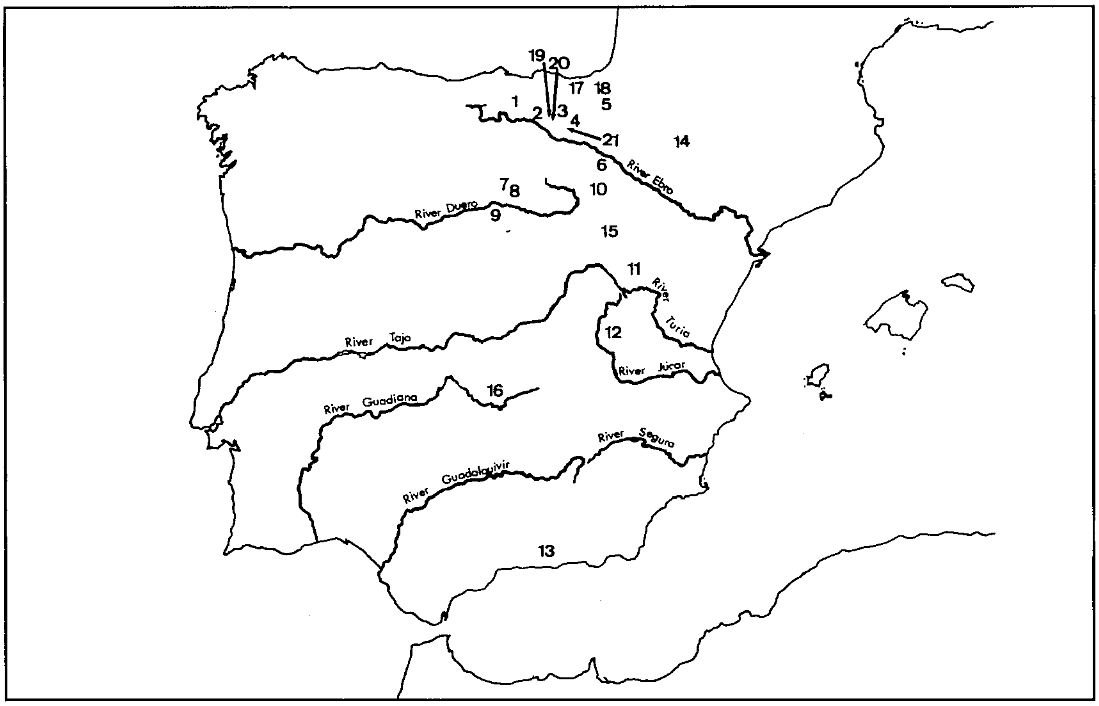


**Figure 6**

**Recent mass mortalities of crayfish populations in Spain. Numbers designate the crayfish populations which are indicated in Table I.**

**Figure 6**

**Nouveaux cas de mortalité massive des populations d'écrevisses en Espagne. Les numéros désignent les populations d'écrevisses répertoriées dans le Tableau I.**

On the other hand, it has often been assumed that the crayfish plague fungus is the only reason of disappearance of the native species. However, it is also shown in this paper that disappearances of crayfish can be due to other reasons such as other diseases, *i.e.*, saprolegniasis, pollution, *etc.* (Table I). The negative effects of other diseases, and pollution have been previously described by other authors (VEY, 1981 ; DIÉGUEZ-URIBEONDO *et al.*, 1994 and TAUGBØL and SKURDAL, 1993). Moreover, it should be taken into account that many of the native populations are dwelling in isolated places where they are very sensitive to environmental changes such as climatic drought, pollution, overfishing, *etc.* Recent studies in Burgos and Navarra (TEMIÑO and SÁEZ-ROYUELA unpublished ; DIÉGUEZ-URIBEONDO *et al.*, 1997 respectively) show that at least one third of the cases of disappearances of *A. pallipes* populations during the period 1985-1996 have been due to climatic drought, pollution, habitat alterations and poaching.

Furthermore, the impact of stressing factors should be also considered since it is known that chronically infected North American crayfish species are subjected to additional stress if factors such as high population density, temperature changes, acidic pH, *etc.*, are no longer capable of defending themselves against the crayfish plague fungus (SÖDERHÄLL, 1988, 1990). This fact was corroborated by the finding of high mortalities in two populations of signal crayfish in the provinces of Guipúzcoa and Navarra (Table I).

The results of this work emphasize even more the necessity of controlling the introduction of alien species in order to control the spread of the crayfish plague fungus. The native species of freshwater crayfish of the Iberian Peninsula is listed in the Red Data Book of the International Union for the Conservation of Nature and Natural Resources (IUCN) as endangered species (GROOMBRIDGE, 1994). Therefore, management plans for native species and alien species urge to be implemented in order to preserve and restore the native crayfish population where possible.

## CONCLUSIONS

**1** The first outbreak of crayfish plague in Spain appears to have been in 1978. Introductions of North American species of crayfish, *i.e.*, *Procambarus clarkii* and *Pacifastacus leniusculus*, could have been the way by which this disease came into the Iberian Peninsula.

**2** Crayfish plague is still causing mortalities of native species *A. pallipes.* These mortalities are related to presence of populations of North American species of crayfish in the vicinity, *i.e. P. leniusculus.*

**3** Other factors are also affecting the native crayfish populations, *i.e.*, saprolegniasis, pollution, habitat alterations, climatic drought, overfishing.

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