

Comparative study between European and American species of *Polistes* using sera from European sensitized subjects

F. SANCHEZ, M. BLANCA, J. FERNANDEZ*, A. MIRANDA, A. TERRADOS†, M. J. TORRES, A. DEL CANO, J. J. GARCIA and C. JUAREZ

Allergy Unit, Internal Medicine Department, Carlos Haya Hospital, Malaga,*Allergy Unit, Hospital Seguridad Social, Elche, Alicante and †Allergy Unit, Hospital Clinico San Cecilio, Granada, Spain

Summary

Although the different vespid species of the genus *Polistes* are found all over the world, studies about allergy sensitization to these species have been made mainly in North America. We studied the specific IgE in a large group of sera from patients sensitized to *Polistes dominulus* (PD) and determined the crossreactivity with another important European *Polistes* (*P. gallicus* [PG]) and with five American relevant species of this genus: *P. exclamans* (PE), *P. fuscatus* (PF), *P. metricus* (PM), *P. annularis* (PAN) and *P. apachus* (PAP). Studies indicated that 96.7% of sera with IgE positive to PD were positive to PG. No case positive to just PG was found. The comparison of these two European species with the American relatives indicated that most cases were positive to all the insects although there were significant differences in the radioallergosorbent test (RAST) value. These results were confirmed by RAST inhibition studies, and indicate that although the European and American species are closely related, species specific allergenic differences exist. These data suggest that *in vivo* studies should be carried out in order to determine the sensitivity and specificity of the European venoms compared with the American species.

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Introduction

The family Vespidae includes three genera: *Vespula thompsoni*, *Vespa* and *Polistes*. There are antigenic and allergenic differences between the species of these genera [1–3]. Although there are common species, different species also exist in Europe and North America. Figure 1 shows the relevant species found in Europe.

Studies of insect sensitivity distribution have been mainly carried out in the United States [4]. Twelve species of yellow jacket have been identified [5]. These seem to have common antigenic and allergenic components although they are species specific components [5].

Yellow jacket and hornet venoms are closely related but *Polistes* wasps are more distantly related and some

yellow jacket allergic patients have a positive skin test to wasp venoms [3].

Early studies indicated that there was little or low crossreactivity between *Polistes* and *Vespula* species [6,7] although recent studies indicate that the degree of cross-reactivity varies according to the different allergenic components of the venom. Homologous antigens 5 and hyaluronidases of vespids show varying degrees of antigenic crossreactivity while phospholipases A show no detectable crossreactivity [8].

Polistes venom sensitivity has been reported to be important in southern parts of the United States from Virginia to Florida and from Arizona to Texas [6,9]. Vespid distribution studies carried out outside the United States have indicated that *Polistes* species are also relevant in other parts of the world [10–13].

European species of *Polistes* are different to those found in America. Thus, we have reported the existence

Correspondence: Professor C. Juarez, Internal Medicine Department, Carlos Haya Hospital, 29010 Malaga, Spain.

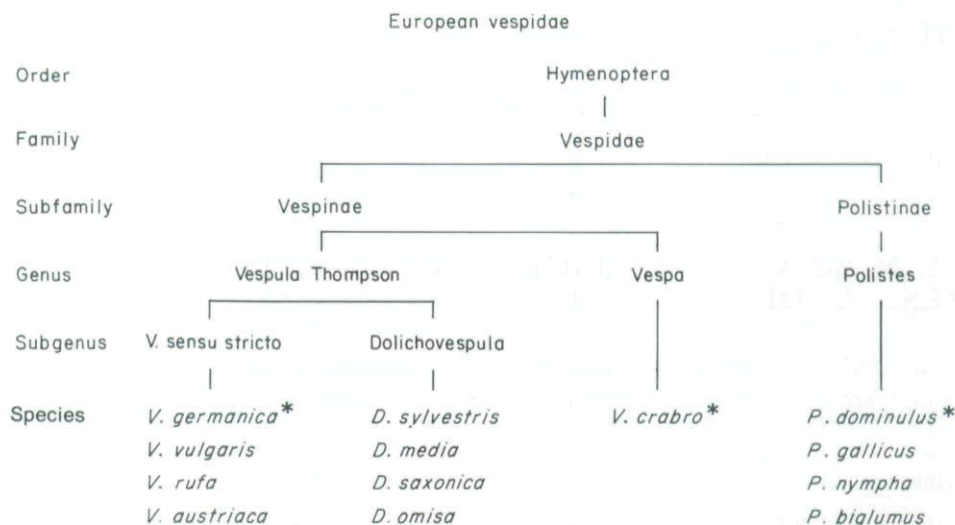


Fig. 1. General classification of species of European vespids. Those marked with an asterisk are the most relevant species found able to induce allergic reactions.

of *Polistes dominulus* (PD), *Polistes gallicus* (PG), *Polistes nympa* (PN) and *Polistes biglumus* (PB) [11]. The first two species (PD and PG) were the most relevant in the induction of allergic reaction [11]. Insect distribution studies in the area where the patients came from indicated that PD was present in 80%, PG in 19%, PN in 0.9% and PB in 0.1% [12,13].

No comparative studies have been undertaken between American and European species of *Polistes* using sera from subjects sensitized in Europe. Studies of sensitization to PD using sera from American subjects have been already reported [14]. In this work a large group of sera was studied in order to learn the distribution of IgE antibodies to PD and PG and the allergenic relationship with five American species of *Polistes* mentioned above: PE, PF, PM, PAN and PAP. Studies indicated that although there was a high correlation between all the venoms, differences did exist, indicating that individual sera may only recognize allergenic determinants present in some species and not in others.

Materials and methods

Venom collection

PD and PG vespids were obtained in local areas where these species are usually found. Insects were immediately frozen in dry ice after collecting and the venom was obtained by the sac dissection method [15]. Sacs were removed and punctured in a Petri dish in beta-alanine buffer. The venoms were then dialysed, lyophilized and kept at -20°C until used. The venoms from the Amer-

ican species (PE, PF, PM, PAN and PAP) were obtained from VESPA Laboratories, INC. (Spring Mills, PA, USA).

Patient selection

Sera from subjects with a clinical history of local or systemic reactions to *Polistes* and with positive skin tests were used in this study. They all had to have IgE antibodies to PD, PG or both.

Protein content and enzymatic activity

Protein concentration in all the venoms was quantitated by the Bradford method [16] and the allergenic activity was estimated for hyaluronidase following the method described by Hoffman and Wood [17] and for phospholipase following the method described by Mulfinger *et al.* [18].

RAST

Cyanogen bromide activated cellulose discs were conjugated to the different venoms at an optional concentration as described [13]. Radiolabelled anti-IgE from Pharmacia (Uppsala, Sweden) was used and the results were presented as the percentage of maximum counts.

RAST inhibition

The allergenic crossreactivity of the different species of *Polistes* was studied by RAST inhibition using in the solid phase discs conjugated to either PD or PG and in

the fluid phase all the venoms used in this study. Inhibition was carried out as described [11].

Statistical studies

The different venoms were compared by the correlation coefficient and the RAST values obtained with all the venoms were compared by analysis of variance.

Results

The comparison of the enzymatic activity of the different venoms is presented in Fig. 2. It can be seen that for hyaluronidase and phospholipase A₁ B the allergenic content of all the venoms was similar for the same concentration. Accordingly, RAST discs for all the studies were conjugated to the different venoms at the concentration of 1 mg/ml. The study included 31 sera. RAST values over a previously established coefficient of variation (CV) of 15% for both PD and PG were considered as different. The comparison of the RAST value carried out in these sera with both PD and PG venoms is presented in Fig. 3. In 22 cases PD was more positive, in two cases PG was more positive and in seven cases there were similar values for both venoms. Except in one serum (number 31) that was negative to PG all the other subjects had IgE antibodies to both venoms. No serum with RAST positive to PG and negative to PD was found. Most of the sera were more RAST positive to PD than to PG.

The RAST values to all the different species included in this study are presented in Table 1. No higher RAST values were obtained with any of the American species than with PD. The analysis of variance showed significant differences between the RAST values of all the different groups with a $P < 0.01$.

The inhibition of four sera of Table 1 (sera 3, 9, 14 and 18) are presented in Fig. 4. It can be observed that in every serum there was a variable degree of crossreactivity with the different venoms indicating that each serum had crossreacting IgE antibodies that recognized different antigenic determinants.

Among the American venoms PAP was the species that produced the highest RAST value in more cases (51.6%), followed by PE (22.7%), PM (16.1%) and PF (6.5%). In all the sera studied the lowest RAST value was given by PAN. Serum number 19 was also negative to all the American species. In 14 cases (45%) the RAST value was higher for at least one of the American species than for PG. Although the correlation value was highly correlated, the relationship between all the species venoms was as follows: PD > PG > PAP > PAN > PF > PM > PE.

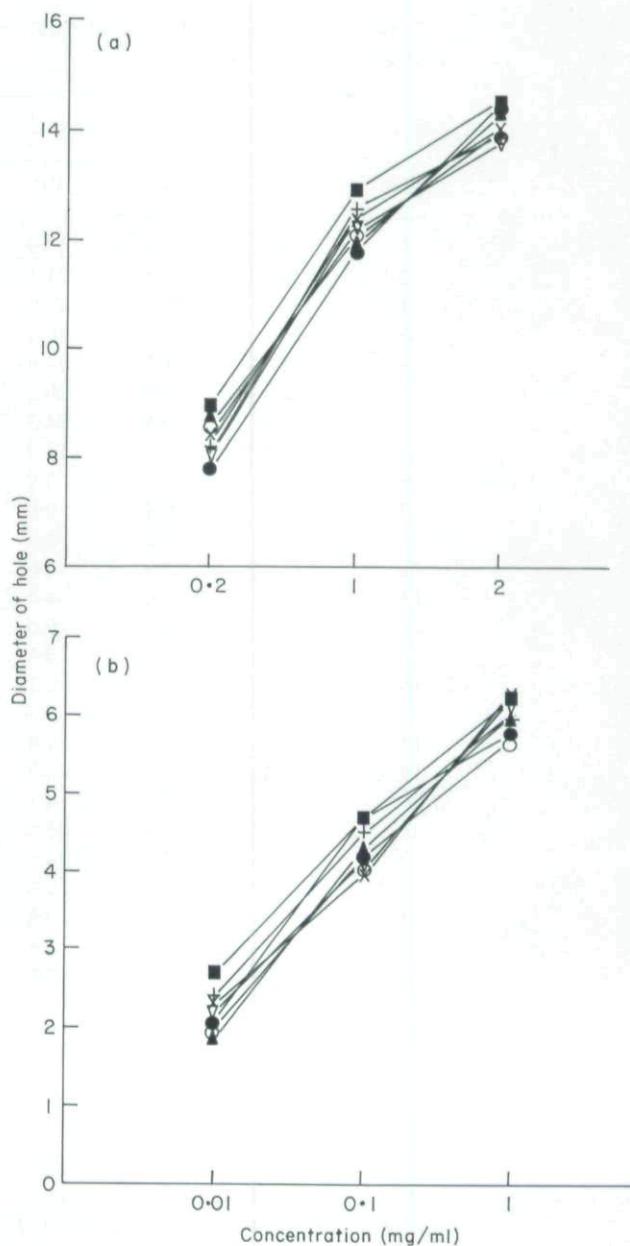


Fig. 2. Study of the hyaluronidase activity (a) and the phospholipase A activity (b) of the venoms studied. Concentrations ranging from 0.2 to 2 mg/ml for hyaluronidase and from 0.01 to 1 mg/ml for phospholipase A₁ B were used. The diameter of the holes produced by the enzymatic activities is presented on the vertical axis in mm. ■, PD. ◆, PG. ▲, PE. ×, PF. +, PM. —, PAN. ▼, PAP.

Discussion

Within the family Vespidae, *Polistes* species are relevant in different parts of the world where they seem to predominate [6,9]. Little information about the distribu-

Table 1. RAST values for the 31 sera using all the species of *Polistes* included in the study

Sera	Venoms						
	PD	PG	PE	PF	PM	PAN	PAP
1	34.9	33.4	17.9	15.3	19.2	18.3	21.2
2	28.5	23.4	21.5	15.7	17.9	16.9	19.9
3	27.2	22.3	21.0	24.7	25.9	11.7	27.16
4	25.7	31.5	10.8	15.4	20.5	8.4	17.0
5	22.9	28.1	14.6	12.4	13.1	10.1	16.8
6	22.8	16.3	10.2	11.5	10.3	8.5	15.7
7	21.4	20.1	14.3	13.0	13.5	3.7	14.1
8	19.6	10.5	17.7	13.6	15.8	8.8	18.4
9	19.3	15.3	5.7	8.2	7.6	4.1	18.7
10	14.8	6.4	9.0	6.0	5.4	7.8	10.7
11	12.6	8.2	4.4	5.7	3.3	6.1	6.8
12	11.0	9.9	4.6	4.7	9.2	3.8	9.2
13	10.9	8.4	4.4	4.7	3.7	3.7	6.5
14	10.3	8.1	0.6	2.9	7.7	2.6	0.8
15	10.1	5.3	4.3	7.0	5.9	4.4	10.0
16	9.9	8.0	9.1	5.5	3.1	6.4	7.9
17	8.1	5.5	3.7	2.9	5.1	1.8	5.2
18	7.9	6.9	7.4	4.1	2.6	6.1	2.9
19	7.4	6.9	2.7	2.2	2.2	2.7	2.0
20	7.2	5.1	4.1	4.4	4.6	4.6	2.5
21	6.7	4.4	6.6	6.2	2.7	1.7	6.4
22	6.4	5.0	4.6	5.4	5.5	1.5	6.3
23	6.3	6.3	0.3	1.7	5.5	1.4	0.5
24	5.4	4.2	2.8	3.4	3.3	2.9	3.3
25	5.1	4.6	1.9	3.8	2.2	2.9	3.1
26	3.7	1.9	3.7	2.1	1.3	1.8	2.8
27	3.0	2.3	2.9	2.5	2.7	1.5	2.9
28	2.1	1.4	1.8	1.2	1.8	1.6	2.1
29	2.1	1.0	0.0	0.0	0.0	0.4	1.5
30	1.9	1.1	1.8	1.9	1.9	1.4	1.8
31	1.3	0.4	0.0	0.0	0.0	0.0	0.0

PD. *Polistes dominulus*. PG. *Polistes gallicus*. PE. *Polistes exclamans*. PF. *Polistes fuscatus*. PM. *Polistes metricus*. PAN. *Polistes annularis*. PAP. *Polistes apachus*.

tion of sensitivities to these insects can be found outside the United States although in some areas these species can be also relevant [10]. Our group has reported that PD and PG are relevant species in the Mediterranean area where in many instances subjects are exposed more to these than to the other species and consequently the percentage of population sensitized to these is higher [11–13,19]. Furthermore, studies have shown that PD is established in the New York and Boston city areas although originally this species was not native to North America [14].

In this work a large group of positive sera from subjects sensitized to PD in Spain was studied in order

to determine the presence of IgE antibodies to two common European species of *Polistes* and also to determine the degree of crossreactivity with five relevant American species of this genus.

The comparison of RAST results between the two European species showed that in more instances sera were more RAST positive to PD than to PG and only in the minority of cases was PG value by RAST higher than PD. These differences were confirmed by RAST inhibition studies and were in agreement with insect distribution studies [12,13].

When RAST value was determined to the American species the sera studied also recognized allergens of these

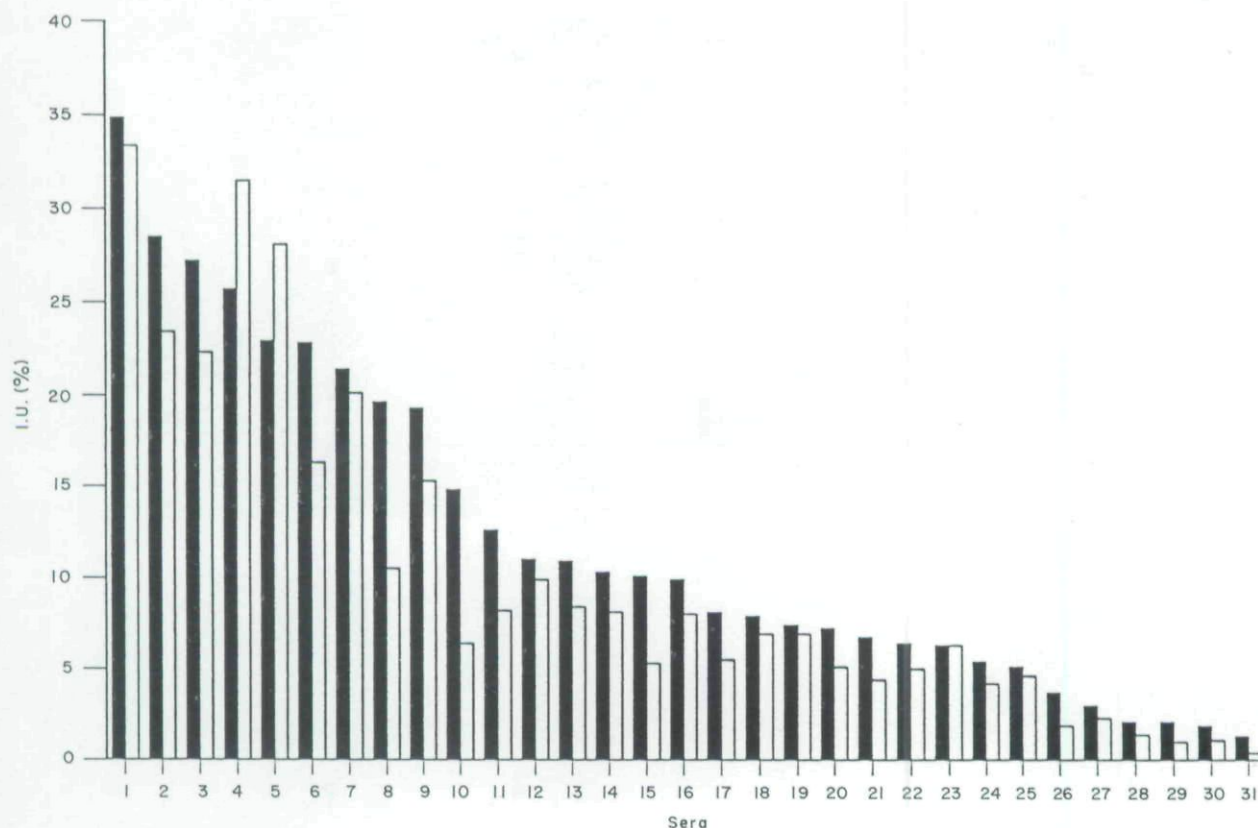


Fig. 3. RAST values to the European species of *Polistes* (PD and PG) of all the sera used. Discs conjugated to the venoms at the optimal concentration of 1 mg/ml were used. Results are expressed as percentage of the maximum counts on the vertical axis. ■. PD. □. PG.

venoms giving values equal to or lower than those obtained with PD and PG venoms. Since American species of *Polistes* do not exist in the area where the study was carried out, this implied that positivity is due to crossreactivity.

As has been reported with other species of vespids [5,20,21] and among *Polistes* species [2,22], although there were common allergens reflected in the positive RAST to all the studied species of *Polistes* in most sera, there were also species-specific allergens as indicated by the statistically significant differences between the RAST value. Differences between the European and the American species of *Polistes* have been reported by Hoffman *et al.* using sera from American subjects sensitized to the American *Polistes* indicating that PD and PG share common allergenic determinants with North American species of *Polistes* but also showing significant allergenic differences [14]. RAST inhibition studies confirmed the direct RAST results obtained in all the sera where slight or striking differences existed.

Among the *Polistes* species found in Europe those

found by our group in the area where these studies were carried out were *P. dominulus* (PD), *P. gallicus* (PG), *P. nynpha* (PN) and *P. biglumus* (PB). Insect distribution studies have shown that of these, the most relevant is PD followed by PG and so far no evidence of IgE positive sera to the other species (PN and PB) has been reported. The distribution of these two last insects indicated that this seems very unlikely although the possibility may exist. The extensive crossreactivity between European and American species indicates that in most instances American species can substitute the European ones when *in vitro* data are considered. However, in *in vitro* studies large quantities of venoms were conjugated to the solid phase in order to achieve maximum sensitivity and all subjects included were skin-test positive to both species. We do not yet know in how many instances subjects sensitive to the European species will be also positive to the American ones by skin testing. Studies in order to answer this question should therefore be carried out.

To our knowledge this is the first work to study the

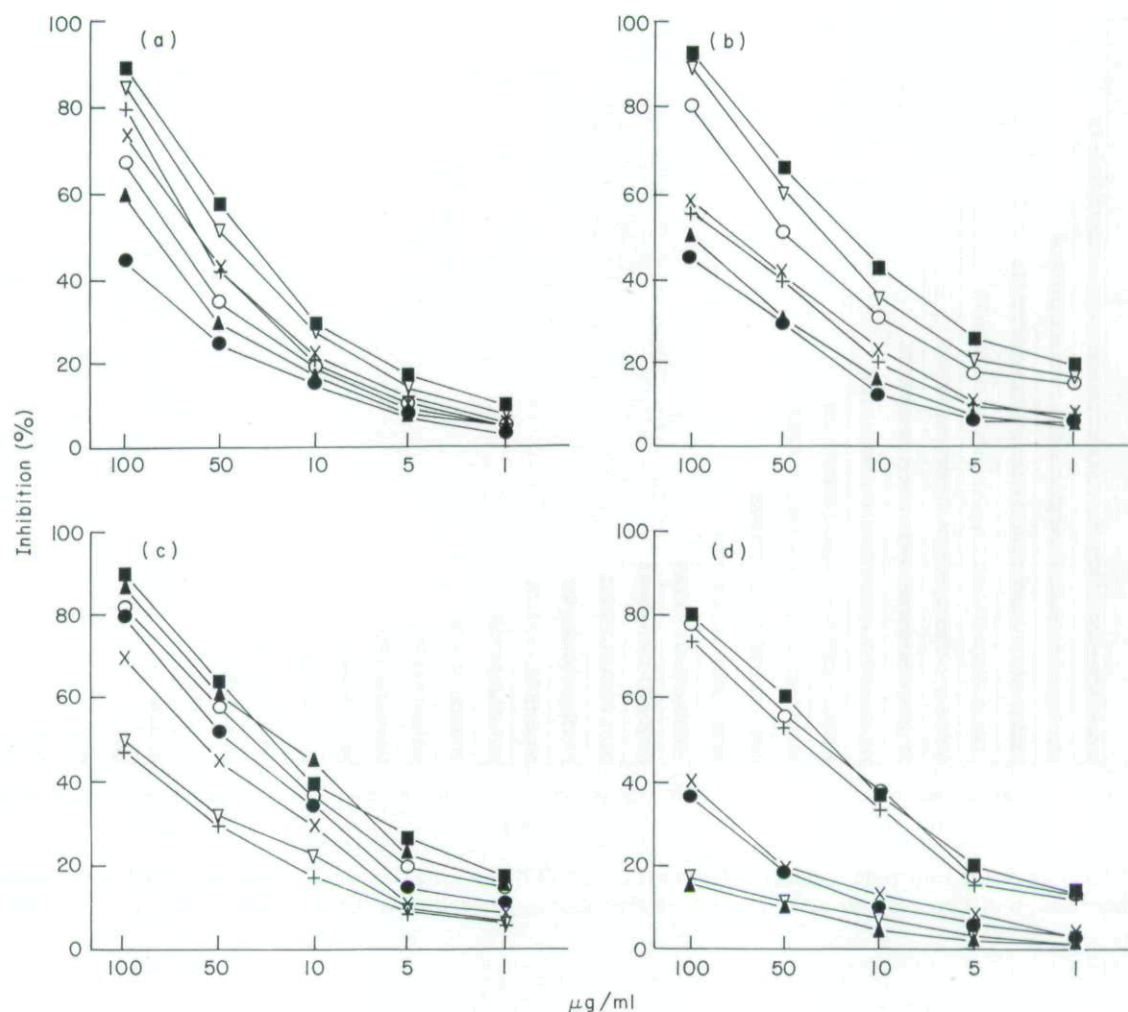


Fig. 4. RAST inhibition studies comparing four sera of Table 1: sera 3, 9, 14 and 18. In the solid phase discs conjugated to PD were used and in the fluid phase all the American and European venoms studied at concentrations ranging from 1 to 100 $\mu\text{g/ml}$ were used. Results are expressed as percentage of inhibition. (a) Serum 3. (b) Serum 9. (c) Serum 18. (d) Serum 14. $\blacksquare$, *P. dominulus*. $\blacklozenge$, *P. gallicus*. $\blacktriangle$, *P. exclamans*. $\times$, *P. fuscatus*. $+$, *P. metricus*. $- \cdot -$, *P. annularis*. $\blacktriangledown$, *P. apachus*.

presence of IgE antibodies to European species of *Polistes* and determine the crossreactivity with different American species using sera from European subjects. In order to learn the distribution of sensitivities to these insects, studies should be carried out in other parts of Europe, especially in those countries where distribution of these species is relevant.

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