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Phospholipase A1-based cross-reactivity among venoms of clinically relevant Hymenoptera from Neotropical and temperate regions

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ABSTRACT

Molecular cross-reactivity caused by allergen homology or cross-reactive carbohydrate determinants (CCDs) is a major challenge for diagnosis and immunotherapy of insect venom allergy. Venom phospholipases A1 (PLA1s) are classical, mostly non-glycosylated wasp and ant allergens that provide diagnostic benefit for differentiation of genuine sensitizations from cross-reactivity. As CCD-free molecules, venom PLA1s are not causative for CCD-based cross-reactivity. Little is known however about the protein-based cross-reactivity of PLA1 within vespid species. Here, we address PLA1-based cross-reactivity among ten clinically relevant Hymenoptera venoms from Neotropical and temperate regions including *Polybia paulista* (paulistinha) venom and *Vespula vulgaris* (yellow jacket) venom. In order to evaluate cross-reactivity, sera of mice sensitized with recombinant PLA1 (rPoly p 1) from *P. paulista* wasp venom were used. Pronounced IgE and IgG based cross-reactivity was detected for wasp venoms regardless the geographical region of origin. The cross-reactivity correlated well with the identity of the primary sequence and 3-D models of PLA1 proteins. In contrast, these mice sera showed no reaction with honeybee (HBV) and fire ant venom. Furthermore, sera from patients monosensitized to HBV and fire ants did not recognize the rPoly p 1 in immunoblotting. Our findings reveal the presence of conserved epitopes in the PLA1s from several clinically relevant wasps as major cause of PLA1-based *in vitro* cross-reactivity. These findings emphasize the limitations but also the potential of PLA1-based HVA diagnostics.

Abbreviations: CCDs, cross-reactive carbohydrate determinants; CRD, component-resolved diagnosis; HBV, honeybee venom; HVA, Hymenoptera venom allergy; PLA1s, phospholipases A1; Poly p 1, phospholipase A1 from *Polybia paulista* venom; PPV, *P. paulista* venom; vPLA1s, vespid phospholipases A1; YJV, yellow jacket venom

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1. Introduction

Hymenoptera venom allergy (HVA) is one of the major causes of anaphylaxis worldwide (27% as compared to drugs and food with 41% and 20% respectively) (Oropeza et al., 2017). In untreated patients, insect stings can provoke IgE-mediated systemic reactions with potentially fatal outcome (Bilò, 2011). Identification of the primary sensitizing species is a critical prerequisite for venom immunotherapy (VIT), the only disease-curative treatment currently available. VIT is mostly well tolerated and reduces the risk of subsequent hypersensitivity reactions in the majority of the patients treated (Ollert and Blank, 2015).

HVA diagnosis is based on the patient's history along with skin tests and *in vitro* detection of specific IgE (sIgE) (Biló et al., 2005). Determination of sIgE however is significantly hampered by cross-reactivity. Up to 75% of double positivity to HBV and YJV are suggested to be caused by sIgE to CCDs (Müller et al., 2009), which are defined by an α1,3-fucose linked to the core glycan found on the majority of HBV and YJV allergens (Spillner et al., 2014). The production of CCD-free recombinant allergens such as vespid antigen 5 s and PLA1s or PLA2 from HBV using *E. coli* and *Spodoptera frugiperda* Sf9 cells significantly helped to overcome CCD-based cross-reactivity (Perez-Riverol et al., 2015; Seismann et al., 2010a; Frick et al., 2016; Köhler et al., 2014). Meanwhile, cross-reactivity due to common epitopes in homologous venom allergens remains a challenge for HVA diagnosis. The similarities of the allergen arsenal in the venom often prevent the species-specific differentiation of the culprit insect (Hoffman, 2008; Schiener et al., 2017). In Europe, protein-based cross-reactivity has particularly hindered the differentiation of YJV and *Polistes* (paper wasp) venom sensitizations (Caruso et al., 2007; Monsalve et al., 2012; Schiener et al., 2017). Species of the Polistinae subfamily have gained interest as they represent a major cause of wasp allergy in some geographical regions and are increasingly spreading to other moderate climate zones. Thus, characterization of allergens and protein-based cross-reactivity is mandatory to increase the specificity of the diagnosis.

In contrast to Europe, a broad diversity of clinically relevant insects has been described for Neotropical regions. Brazilian species (Fig. 1, Supplementary data Fig. 1) comprise 33% of the currently identified wasp species worldwide (Locher et al., 2014). In Brazil, members of Polistinae subfamily have been also reported as the major cause of sting accidents and insect-related anaphylaxis (Perez-Riverol et al., 2017). The venom composition of these Hymenoptera however has been poorly characterized and recombinant venom allergens from endemic species are not available for routine diagnosis (Bazon, 2017; Perez-Riverol et al., 2016). Therefore, diagnostics are usually based on non-commercial crude venoms or reagents from Northern Hemisphere species like *Vespula vulgaris* (Perez-Riverol et al., 2017). The use of such material for diagnosis is associated with low sensitivity, high levels of CCD or peptide-based cross-reactivity and thus unreliable characterization of the patienťs sensitization profile.

To date, mainly the species-specific marker allergens antigen 5 (Ves v 5) and PLA1 (Ves v 1) from YJV as well as PLA2 (Api m 1) from HBV have been established for the differentiation of genuine sensitization to HBV and YJV (Korošec et al., 2012; Monsalve et al., 2012; Seismann et al., 2010b). Differentiation of wasp allergic patients however remains challenging. Venom PLA1s have been proven valuable tools as they allow the detection of sensitization in patients with negative sIgE to antigen 5 (Ebo et al., 2012; Korošec et al., 2012; Seismann et al., 2010b; Sturm et al., 2010). Moreover, the lower identity among wasp venom PLA1s as compared to antigens 5 (Monsalve et al., 2012; Schiener et al., 2017) renders PLA1s promising tools for identification of culprit species.

Here, we analyzed the PLA1-based cross-reactivity of venoms from eight Hymenoptera of clinical relevance in South America and two of their European counterparts. Our results suggest that PLA1s might be useful indicator allergens for differential sIgE diagnosis of vespid venom allergy within Neotropical regions and as marker allergens for differentiation of sensitization to wasp/honeybee and wasp/ant venoms.

2. Materials and methods

2.1. Allergic patients' sera

Sera from allergic patients monosensitized to *P. paulista* (n = 4), honeybee (n = 12) or fire ant (n = 4) venom and previously diagnosed by ELISA or ImmunoCAP 250 (Phadia, Upsala, Sweden), regardless sex or age, were obtained from the Ambulatório de Anafilaxia of the Hospital das Clínicas (Universidade Estadual de Campinas-UNICAMP,

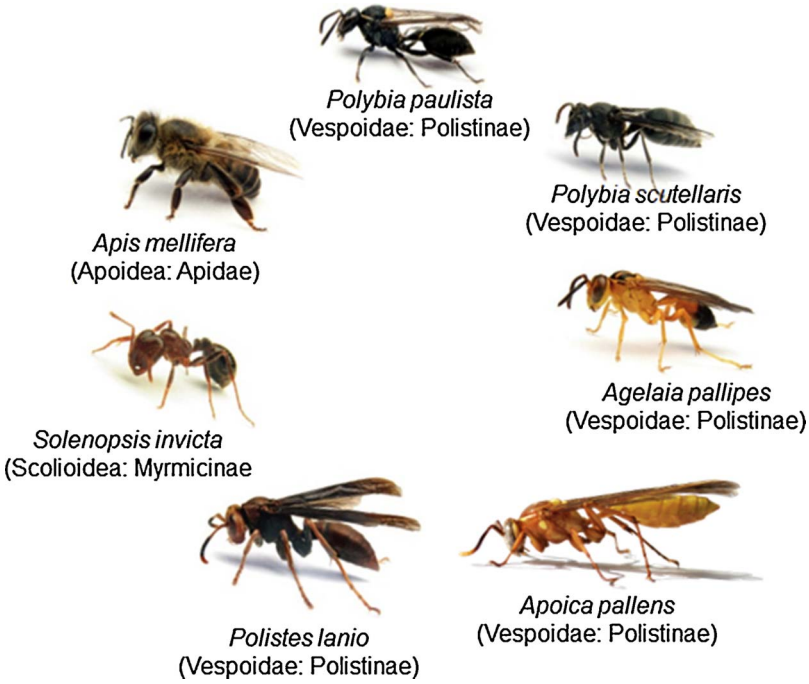

Fig. 1. Clinically relevant species of social Hymenoptera from Brazil. (Photos kindly provided by Professor M.S. Palma).

Campinas, Sao Paulo State, Brazil). Two sera from non-sensitized healthy volunteers were used as negative controls. The study was approved by the Ethics Committee of FCM-UNICAMP under n° 187/2006 (23rd July, 2006, updated in 2008). Informed consent was obtained in written form from all participants of the study.

2.2. Insect venoms and allergens

Nests from wasp species (*P. paulista, P. ignobilis, P. scutellaris, P. lanio, A. pallens and A. pallipes*) were captured around or within the campus of Universidade Estadual Paulista "Júlio de Mesquita Filho" (UNESP), Rio Claro, SP, Brazil (Licensed by Sisbio, No. 58500). *A. mellifera* individuals were obtained from colonies kept in the vivarium of UNESP's Rio Claro Institute of Biosciences. Venom extracts, native Poly p 1 and rPoly p 1 were obtained using previously described procedures (Perez-Riverol et al., 2016). PLA2 (Api m 1) and mellitin (Api m 4) were obtained commercially (Sigma, US). Venom extract from *S. invicta* was kindly provided by Prof. R.L. Zollner from the stock at the Laboratory of Translational Immunology (UNICAMP, Campinas, SP, Brazil). Venoms and PLA1s from *P. dominula* and *V. vulgaris* were provided by Euroimmun (Germany).

2.3. Generation of monospecific sera by immunization

Five Balb/c mice (female, 4 weeks old) were immunized intradermally with 20 μg of rPoly p 1 in PBS/Al$(OH)_3$ in six weekly doses. Three mice were immunized with 20 μg of solubilized proteins from *E. coli* BL21 (DE3) transformed with pET-28a vector for control purposes. A description of these protocols is given in the Supplementary Data.

2.4. Immunoblotting and ELISA

The sIgG and sIgE-mediated recognition of venoms and allergens by patients' sera and/or by sera from rPoly p 1-sensitized mice were assessed by immunoblotting and/or ELISA using protocols previously described (Perez-Riverol et al., 2016; Schiener et al., 2017). These protocols are provided in the Supplementary Data. The data analysis relied on Microsoft Excel, version 14.0 (Microsoft, US).

2.5. Molecular modelling

The 3-D models of PLA1s were generated by MODELLER (Martí-Renom et al., 2000) using the structure of the venom PLA1 from *Vespa basalis* (PDB ID: 4QNN) as general template. The model was subjected to energy minimization using YASARA software (Krieger et al., 2009) and then validated using MolProbity (Chen et al., 2010) The ribbon diagram was prepared with PyMOL using the homology models as input.

3. Results

3.1. Recombinant Poly p 1- mediated sensitization of mice

The capability of rPoly p 1 to induce *in vivo* sensitization of mice (n = 5) after intradermal immunization was evaluated by immunoblotting. Detection of sIgE showed that allergen-specific reactivity with nPoly p 1 (Fig. 2a) and with *P. paulista* venom (Fig. 2b). A unique band corresponding to the predicted molecular weight of Poly p 1 (~34 kDa) was obtained in immunoblotting with purified nPoly p 1 and with the positive control (rPoly p 1) (Fig. 2a, d). For the crude venom, a second and less intense band (~25 kDa) was observed (Fig. 2b). The absence of detection for purified native Poly p 5 (50 μg) (Fig. 2c) which has a similar molecular weight (dos Santos-Pinto et al., 2014) suggests that the band corresponds to one of the multiple form of Poly p 1 identified in *P. paulista* (dos Santos et al., 2011) or a degradation product present in the venom.

3.2. Cross-reactivity of sera from rPoly p 1-sensitized mice

Relevant non-vespid Hymenoptera species in Neotropical regions mainly are honeybee and fire ant. Assessing the sera from rPoly p 1-sensitized mice in immunoblotting, we did not observe any cross-reactivity with neither HBV allergens (Api m 1, Api m 4) nor crude venom extract (Fig. 3a). Similarly, no reactivity was detected with crude venom of *S. invicta* (Fig. 3b). ELISA analyses for detection of cross-reactive sIgE/sIgG to these venoms further corroborated the lack of cross-reactivity (Fig. 5).

Assessment of cross-reactivity to venoms of clinically relevant wasps from Neotropical (Fig. 4a) and temperate regions (Fig. 4b) was performed by detection of IgE and IgG. Notably, IgE as well as IgG reactivity could be detected for all venoms from Neotropical wasps and *P. dominula*, a clinically relevant wasp from Northern Hemisphere. The highest levels of cross-reactivity were obtained with venoms of the taxonomically more related species *P. scutellaris* and *P. ignobilis*. A pronounced reactivity was also detected for venoms from Neotropical species of different genera such as *P. lanio*, *A. pallipes*, *A. pallens* and with the venom from *P. dominula* (Fig. 4b). No reaction however was detected with *V. vulgaris* venom, the most relevant wasp in Europe.

ELISA analyses supported the cross-reactivity among venoms from wasps of Neotropical and temperate regions (Fig. 5, Supplementary data Figs. 3 and 4) using Poly p 1-specific sera. For both IgE and IgG the results were similar to those obtained in the immunoblotting with higher reactivities of the more closely related *Polybia* species, *P. scutellaris* and *P. ignobilis*. Interestingly, high levels of cross-reactivity were also detected for *Polistes* venoms, with *P. dominula* showing a slightly higher signal than *P. lanio* among all individual serum (Supplemantary Data, Figs. 3 and 4). Similar to the immublotting analysis, no cross-reactivity was detected with *A. mellifera, S. invicta, V. vulgaris* venoms and rVes v 1. Against the lack of CCDs (Seismann et al., 2010b), the PLA1-based cross-reactivity indicates the presence of common protein epitopes among the different variants of the allergen in the venoms evaluated here. In order to address the molecular basis of cross-reactivity we analyzed the identity of the primary sequence and structural similarity based on 3-D models of venom PLA1s for those of which the information is available.

Lacking crystallographic data for the PLA1s, the 3-D models of rPoly p 1 (GenBank ID: ADT89774.1), nPoly p 1 (A2VBC4.1), Pol d 1 (AAS67041.1), Ves v 1 (AAB48072.1) and Sol i 1 (NP_001291510) were built using the deposited pdb (PDB ID: 4QNN) of the PLA1 atomic coordinates from the hornet *Vespula basalis*. The lack of sequence information for PLA1s from *P. scutellaris*, *P. ignobilis*, *P. lanio*, *A. pallipes* and *A. pallens* hampers similar structural evaluations. The identity of the rPoly p 1 primary sequence with these PLA1s ranged from 36% (Sol i 1) to 74% (Pol d 1) (Fig. 6). Meanwhile, in the structural analysis the root-mean square deviation (RMSD) of the atomic positions of the 3-D models varied from 0.172-0.083, with Sol i 1 and Pol d 1 showing the lowest and highest identity, respectively. The results from the sequences alignment (Perez-Riverol et al., 2016) and the overlay of the 3-D models (Fig. 6) support the cross-reactivity observed in immunoblotting and ELISA. Higher values of sequence identity and structural similarity are associated with stronger IgE and IgG-mediated recognition of the PLA1s in the insect venoms. The results of different approaches are summarized in Table 1.

3.3. Cross-reactivity of sera from allergic patients

To reassess the potential of rPoly p 1 to differentiate double sensitization to wasp/bee or wasp/fire ant venoms from cross-reactivity, we evaluated the recognition of rPoly p 1 (Fig. 7) by sera from patients sensitized solely to HBV (12), fire ants (4) or *P. paulista* venom. Notably, none of the sera with sIgE to HBV showed cross-reactivity with rPoly p 1. In contrast, all sera of patients sensitized to PPV recognized the rPoly p 1. In summary, these data clearly suggest that the limited cross-

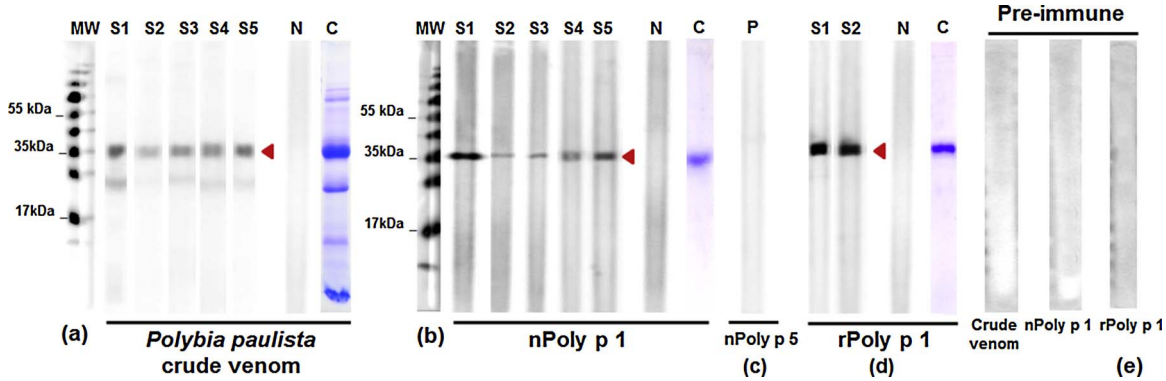

Fig. 2. IgE-mediated immune reactivity of sera from rPoly p 1-sensitized mice against (a) *P. paulista* crude venom, (b), nPoly p 1, (c) rPoly p 5 and (d) rPoly p 1. Samples: S1–S5 = mouse individual serum; P = pool of mice sera (n = 5); N = pool of sera from mice (n = 3) immunized with *E.coli* BL21 (DE3) cells lysate (negative control); C = Coomasie blue stained SDS-PAGE of *P. paulista* crude venom, purified nPoly p 1 and rPoly p 1. The molecular weight marker (kDa) is indicated. (e) Analysis of immunoreactivity of pooled pre-immune sera was performed using venom, nPoly p 1 and rPoly p1 as before.

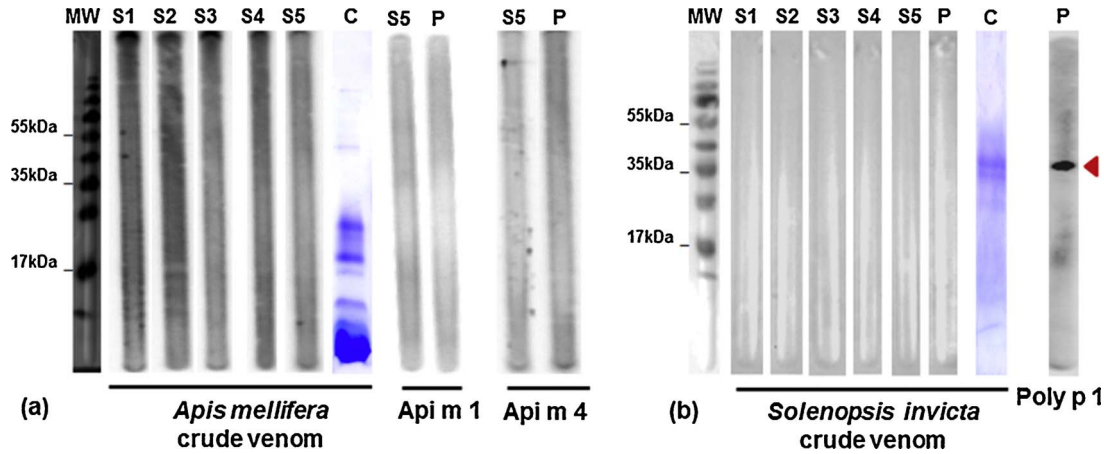

Fig. 3. Analysis of IgE-mediated cross-reactivity of sera from rPoly p 1-sensitized mice with *A. mellifera* venom,Api m 1, Api m 4 (a) and *S. invicta* crude venom (b). Samples: S1–S5 = mouse individual serum; P = pool of mice sera (n = 5); S5 = serum 5; C = Coomasie blue stained SDS-PAGE gel of *A. mellifera* and *S. invicta* crude venoms. The molecular weight marker (kDa) is indicated.

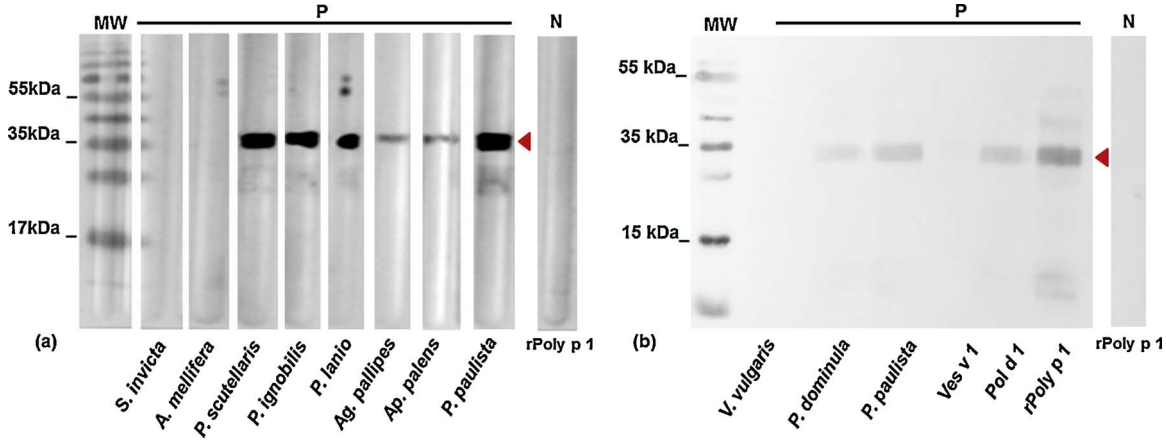

Fig. 4. PLA1-based cross-reactivity (sIgE) among Hymenoptera venoms from Neotropical (a) and temperate regions (b) analyzed by Western blot. Samples: P = pool of sera from rPoly p 1-sensitized mice (n = 5). N = pool of sera from mice (n = 3) immunized with *E.coli* BL21 (DE3) cells lysate (negative control). The proteome profiles of the vespid venoms tested in this study are shown in the Supplementary data, Fig. 2.

reactivity of the PLA1 from *P. paulista* with PLAs from HBV and ant venom renders rPol p 1 a marker allergen for differentiation of sensitization to HBV and ant venom from venom of Neotropical wasps.

4. Discussion

Identification of the primary sensitizing insect is hampered by molecular cross-reactivity associated to the presence of CCDs (Brehler et al., 2013; Seismann et al., 2010a) and common epitopes from homologous allergens such as hyaluronidases (Jin et al., 2010), dipeptidylpeptidases (Blank et al., 2010) and vitellogenins (Blank et al., 2013). Differentiation of hymenoptera belonging to the same family, e.g. Vespula and Polistes species, demands even more refined analysis and knowledge on the molecular characteristics of venom allergens.

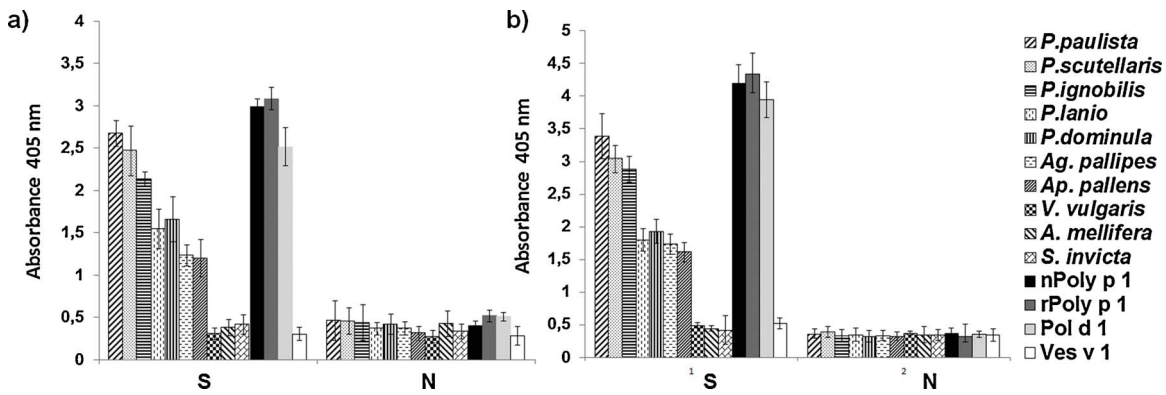

Fig. 5. Detection of cross-reactive sIgE (a) and sIgG (b) in serum of rPoly p 1-sensitized mice in ELISA. S: mouse serum (S5) with the highest levels of anti-rPoly p1 antibodies (IgE and IgG). N = negative control (serum from mouse immunized with a cell lysate of *E.coli* BL21 (DE3) transformed with an empty pET-28a plasmid). The data are the mean of three experimental replicates.

Wasp venom PLA1s are often non-glycosylated and therefore CCD-lacking major allergens without homologues in HBV (Seismann et al., 2010b). These features render this group of allergens a feasible marker for differentiation of wasp and HBV allergy and a candidate for analysis of sensitization to different vespid species. However, little is known about the PLA1-based cross-reactivity among clinically relevant wasps or ants.

In this study, we performed a comprehensive analysis of the insect venom PLA1s-based cross-reactivity among venoms from insects of Neotropical and temperate regions. To address the insect venom PLA1-based cross-reactivity with a molecularly well defined and highly specific tool, we immunized mice with rPoly p 1. The recognition of nPoly p 1 by these sera indicates that the recombinant protein retains the relevant B-cell epitopes from the native form of the allergen. The presence of a second band in immunoblotting might be related to the recognition of a less predominant isoform of the PLA1 at ~25 kDa (dos Santos et al., 2011, 2010)., but proof needs to be provided.

Notably, sera of rPoly p1-sensitized mice did not show cross-reactivity with honeybee venom (and selected HBV allergens) and fire ant venom. These findings support previous results indicating that venom PLA1s are reliable markers for differentiation of wasp or HBV sensitizations (Korošec et al., 2012; Müller et al., 2012; Seismann et al., 2010b).

Immunoblotting and ELISA analyses independently revealed that

Table 1
Summary of the results obtained for PLA1s from venoms of clinically relevant wasps.

rPoly p 1				
Vespid PLA1s	Sequence identity	RMSD	Relative intensity on immublotting reaction	ELISA (OD_{405}nm)[a]
P. paulista	71%	0.080	+ + +	2.55
P. scutellaris	n.a		+ + +	2.41
P.ignobilis	n.a		+ + +	2.24
P. lanio	n.a		+ +	1.53
P.dominula (Pol d 1)	74%	0.083	+ +	1.79
A. pallipes	n.a		+	1.20
A. pallens	n.a		+	1.17
V. vulgaris (Ves v 1)	54%	0.123	−	0.65
S. invicta (Sol i 1)	36%	0.172	−	0.44
A. mellifera	no venom PLA1		−	0.39

n.a: no available in nucleotide/protein databases.
−: negative.
[a] Data from the mouse serum with the highest sIgE levels to rPoly p 1.

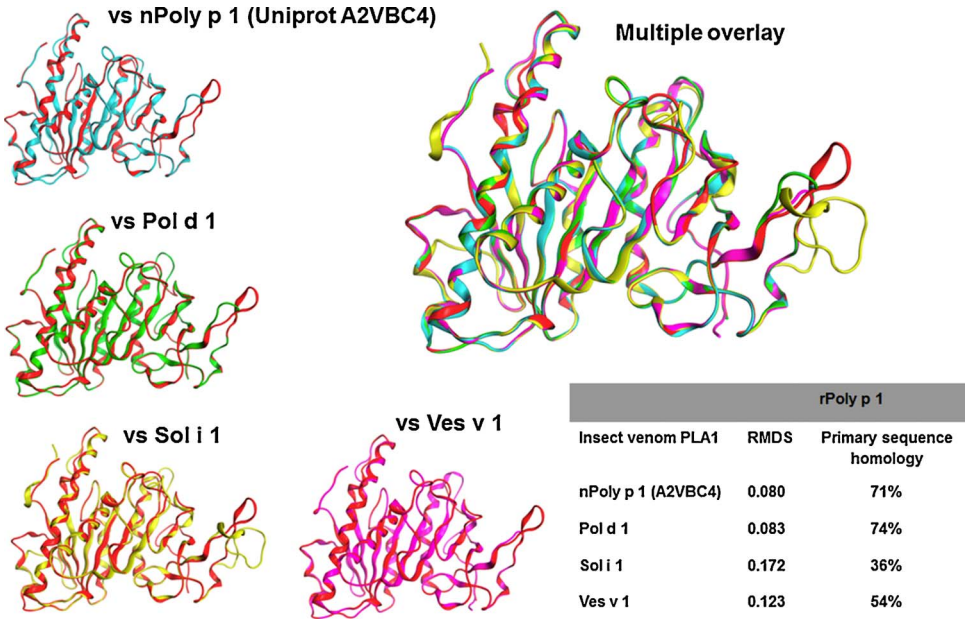

Fig. 6. Overlay of the 3-D models of selected venom PLA1s with the model of rPoly p 1. Samples: rPoly p 1 (red), nPol y p 1 (Uniprot A2VBC4): variant of the native Poly p 1(Santos et al., 2007) (blue), Pol d 1 (green), Sol i 1 (yellow), Ves v 1 (purple). The primary sequence identity and RMSD of atomic positions of the 3-D models from the venom PLA1s compared to rPoly p 1 is shown. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

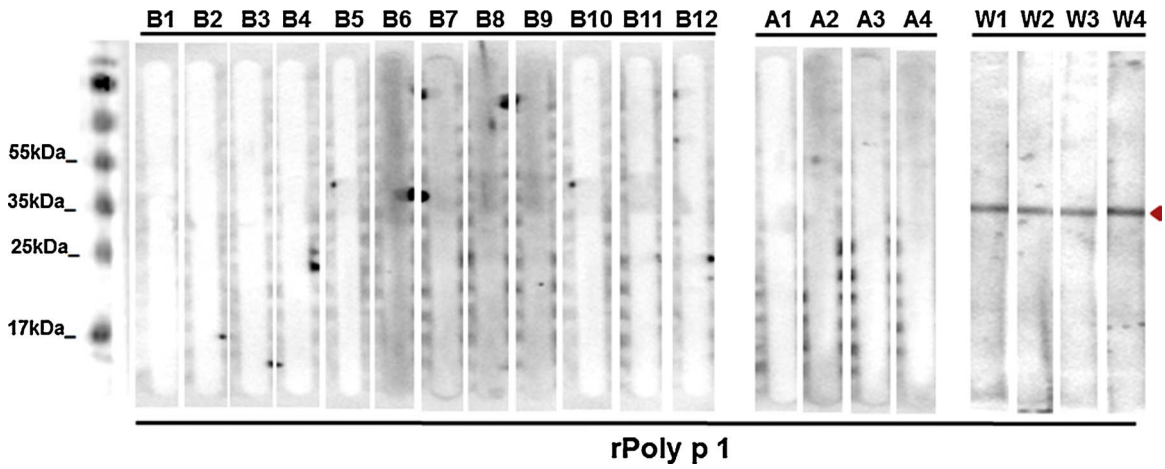

Fig. 7. Cross-reactivity analyses of sera from Brazilian allergic patients. Samples: sera from patients with sIgE to honeybee (B1–B12), fire ant (A1–A4) or *P. paulista* (Control: W1–W4) venoms. The molecular weight marker (kDa) is indicated.

the levels of cross-reactivity among the venom PLA1s from insects of Neotropical zones and with species of temperate regions correlates with the similarity of the primary and 3-D structures. While in HBV a PLA1 has not been identified, Sol i 1 and Ves v 1 showed the lowest values of structural identity with Poly p 1. These findings corroborate the absence of recognition by the sera. Interestingly, cross-reactivity between Ves v 1 and Pol d 1 from *P. dominula* has been reported (Monsalve et al., 2012) indicating molecular similarity between *Vespula* and *Polistes* species. It is important to mention that *Polistes* and *Polybia* species belong to the sub-family of Polistinae (Müller, 2010; Perez-Riverol et al., 2017). Hence, the high level of identity for Pol d 1 was associated with a high cross-reactivity. Unfortunately, sequence data for venom PLA1s from other Neotropical species tested is currently not available.

Several venom PLA1s from species of the genus *Vespula* showed up to 95% of homology (Monsalve et al., 2012). The extensive cross-reactivity obtained for *Polybia* species is likely to be due to high levels of identity on primary sequences and 3-D structures. For *P. scutellaris*, it has been shown that the sequence of antigen 5 (Poly s 5) has a 99% of identity with it counterpart in *P. paulista* venom (Vinzón et al., 2012, 2010). A similar situation might be true for PLA1 from *P. scutellaris* and *P. ignobilis*. The alignment of the primary sequence and 3-D model of the venom PLA1 from *P. dominula*, which is the major European counterpart of the Neotropical wasp *P. lanio* showed a high identity with Poly p 1. Interestingly, the levels of cross-reactivity were similar for *P. dominula* and its Brazilian counterpart, suggesting that venom PLA1 from *P. lanio* could also have high levels of identity with Poly p 1, and retains the homologues IgE-epitopes present in Poly p 1 and Pol d 1. Notably, the identities of Poly p 1 with Pol d 1 and the *P. annularis* PLA1, Pol a 1, are 74% and 71% (Perez-Riverol et al., 2016), respectively. In addition, there seems to be cross-reactivity between the PLA1s from European and North American *Polistes* species. This data suggests a similar level of cross-reactivity between rPoly p 1 and Pol a 1.

Our results showed that vespid PLA1s are promising tools to differentiate wasp/bee and wasp/ant allergy. In contrast, the use of PLA1s for identifying the culprit wasp species is limited by protein-based cross-reactivity. Quantitative assays, however, could provide sIgE levels to different PLA1s and an immanent measure to rank the probability of culprit species. First attempts to differentiate Polistes and Vespula sensitization suggest a feasibility of comparative sIgE determinations (Monsalve et al., 2012).

Diagnosis based on venom extracts is common practice in Latin America and often causative for misidentification of the culprit insect. However, when assessing the IgE reactivity of Brazilian patienťs sera monosensitized to HBV and ant venom (negative on YJV) with rPoly p 1, we did not observe any cross-reactivity. These results emphasize that rPoly p 1 represents a feasible marker for differentiation of wasp sensitization from cross-reactivity with bee and ant venoms in Brazilian patients. Future studies involving a large number of sera are required to verify these initial results. Cellular analyses such as basophil activation tests (Balzer et al., 2014) might further broaden the potential for rPoly p 1- and PLA1-based molecular diagnostics for Neotropical HVA.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.molimm.2017.11.007.

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Supplementary data S1

Phospholipase A1-based cross-reactivity among venoms of clinically relevant Hymenoptera from Neotropical and temperate regions

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1 Supplementary data Material and Methods

1.1 Immunoblotting analyses

For the analyses of the PLA1-mediated cross-reactivity among Neotropical species, proteins were subjected to (12%) SDS-PAGE and then transferred to a 0.22 mm nitrocellulose membrane using a semi-dry system (Trans-Blot® SD Semi-Dry Electrophoretic Transfer Cell, Bio-Rad, US). Prior to the addition of sera from patients or rPoly p 1-sensitized mice, membranes were blocked 2 hours at room temperature with 4% non-fat dry milk in TBS buffer (20 mM Tris-HCl 150 mM NaCl; pH 7.4). After washing (3 times with TBS containing 0.05% Tween), the membranes were incubated overnight at 4 °C and 150 rpm with patients' serum (1:50) or a pool of mice sera (1:200) diluted in TBS buffer with 2% non-fat dry milk. For analyses with patient sera the immunodetection was performed using anti-human IgE (ε-chain specific) peroxidase conjugated antibody (Sigma Aldrich, US) diluted 1:5000 (TBS and 2% non-fat dry milk). For mice sera the membrane was washed and the incubated with a rat anti-mouse IgE (BD Biosciences, Brazil) (1:500) for 2 hours at room temperature followed by incubation with a goat anti rat IgG peroxidase conjugate (Sigma Aldrich, US). The bands were visualized in Image Quant 400 (GE Healthcare, Sweden) using the chemiluminescent substrate Luminata ™ Forte Western HRP substrate (Millipore, USA). For immunoblot analyses with venoms and allergens from European species mice sera was incubated overnight, followed by incubation with a goat anti-mouse IgE antibody (icllab, US) (1:250) and incubation with a rabbit anti-goat IgG alkaline phosphatase conjugated antibody (Sigma, Denmark) (1:20000). The detection of bound antibodies then was performed using BCIP/NBT Color Development Substrate (Sigma, Denmark).

1.2 ELISA

For ELISA analyses, 384-well microtiter plates (Nunc, Thermo Fisher Scientific, Germany) were coated with 20 ng of venoms and purified allergens (4 °C, overnight). Coated plates were then blocked with 4% nonfat dry milk powder (AppliChem, Darmstadt, Germany) in TBS at room temperature for 1h. After washing 3 times with TBS individual sera diluted 1:100 (TBS and 2% nonfat dry milk powder) were added to the plates and incubated overnight at 4 °C. After washing, either a goat anti-mouse IgG conjugated to alkaline phosphatase (1:30000) or a goat anti-mouse IgE (icllab, US) (1:250) diluted in TBS and 2% nonfat dry milk powder were added and incubated by gentle shaking for 1 and 2 hours at room temperature, respectively. For sIgE, a secondary rabbit anti-goat IgG (Sigma Aldrich, US) (1:20000) conjugated to alkaline phosphatase was added (1 hour, gently shacking at room temperature). Detection was performed by adding 50 µL of the substrate solution (5 mg/mL 4-nitrophenylphosphate; AppliChem, Germany). The absorbance was read at 405 nm. An optical density (OD) value that duplicates the means of the negative control was interpreted as positive result. Three independent expreriments for each type of antibodies were conducted.

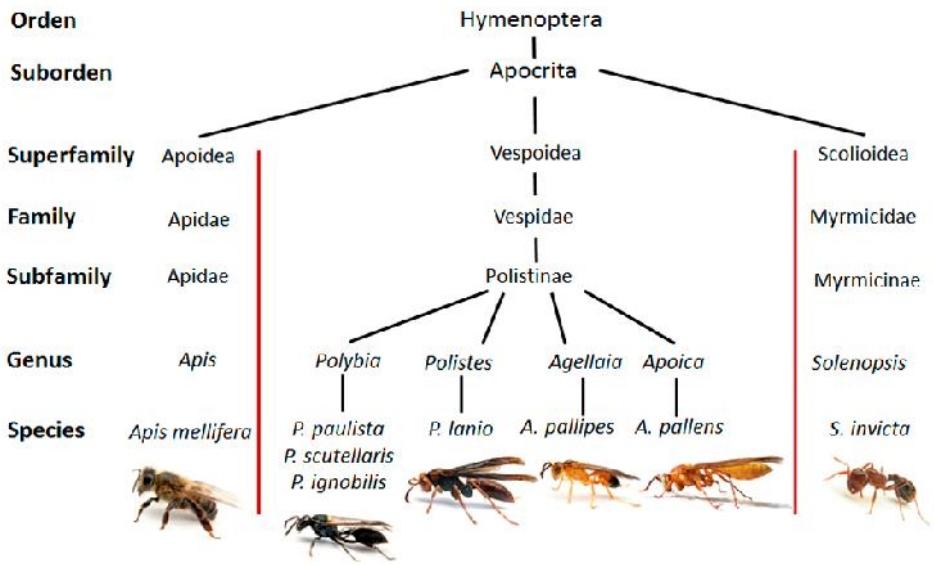
1.3 Polyacrylamide gel electrophoresis and protein quantification

SDS-PAGE was performed using a Mini-Protean® Tetra Cell System (BioRad). After running, the gels were stained with Coomassie Brilliant Blue R-250 (CBB). Protein fractions and purified natural or recombinant allergens were quantified using the modified Bradford method and bovine serum albumin (Sigma, US) as a standard.

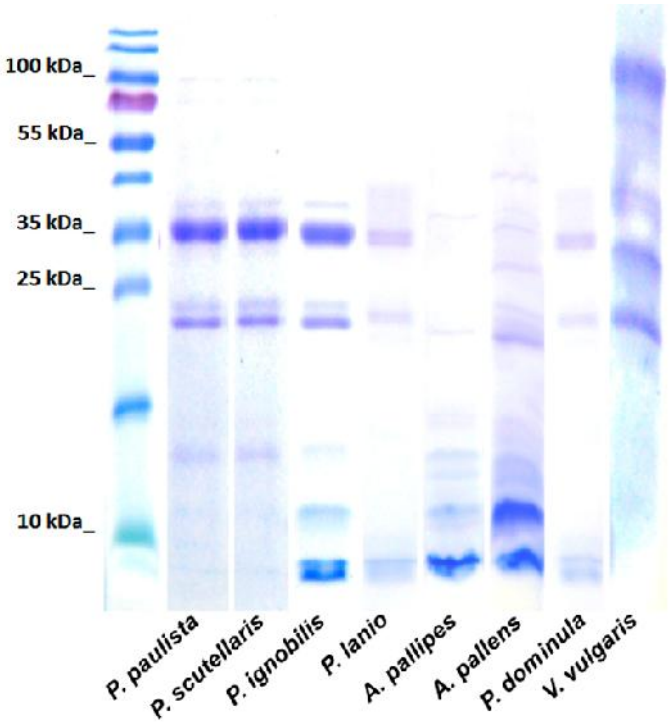
1.4 Generation of monospecific sera by immunization

Specific Pathogen Free (SPF) female BALB/c mice (four weeks old) were obtained from the Multidisciplinary Center for Biological Research (CEMIB) of the University of Campinas (UNICAMP) Campinas, SP, Brazil. They were maintained in SPF environment at 24° C ±1 and photoperiod of 12/12 hours, and fed with autoclaved Nuvilab CR-diet and water ad libitum for at least four weeks before being used in experiments. The methods described in this manuscript were carried out in accordance with the 'Guide for the Care and Use of Laboratory Animals', as promoted by the Brazilian College of Animal Experimentation (COBEA), and was approved by the Ethics Committee for Animal Experimentation of University of Campinas. (CEUA/UNICAMP. Protocol #3476-1). The mice were immunized intradermally with six weekly doses of 20 µg of rPoly p 1 in PBS 5% $Al(OH)_3$. Three mice were immunized with 20 µg of solubilized proteins from *E. coli* BL21 (DE3) cytoplasmatic content transformed with an empty pET-28a vector for control purposess. Seven days after the last immunization, the mice were bled and the sera separated and stored at -20°C.

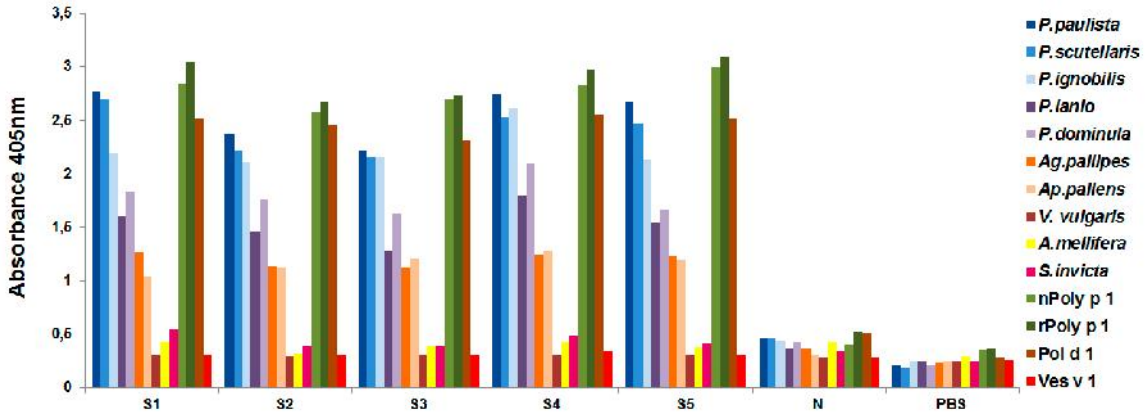
2. Supplementary data Figures:



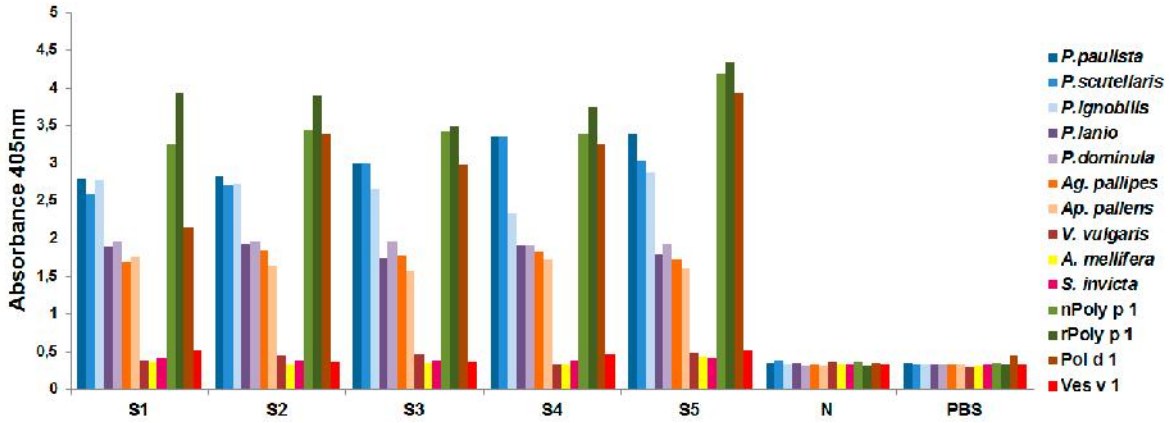
Data S1 Figure 1. Taxonomy of the clinically relevant Neotropical insects including in the study



Data S1 Figure 2. SDS-PAGE analysis of the venom extracts obtained from vespids of Neotropical and temperate regions. The molecular weight marker (kDa) is indicated.



Data S1 Figure 3. Detection of cross-reactive sIgE in the sera from rPoly p 1-sensitized mice measured by ELISA. S: individual mouse serum. N= negative control (serum from mouse immunized with a cell lysate of *E.coli* BL21 (DE3) transformed with an empty pET-28a plasmid). PBS: reagents control. The results represent the media of three replicates of the experiment.



Data S1 Figure 4. Detection of cross-reactive sIgG in the sera from rPoly p 1-sensitized mice measured by ELISA. S: individual mouse serum. N= negative control (serum from mouse immunized with a cell lysate of *E.coli* BL21 (DE3) transformed with an empty pET-28a plasmid). PBS: reagents control. The results represent the media of three replicates of the experiment.