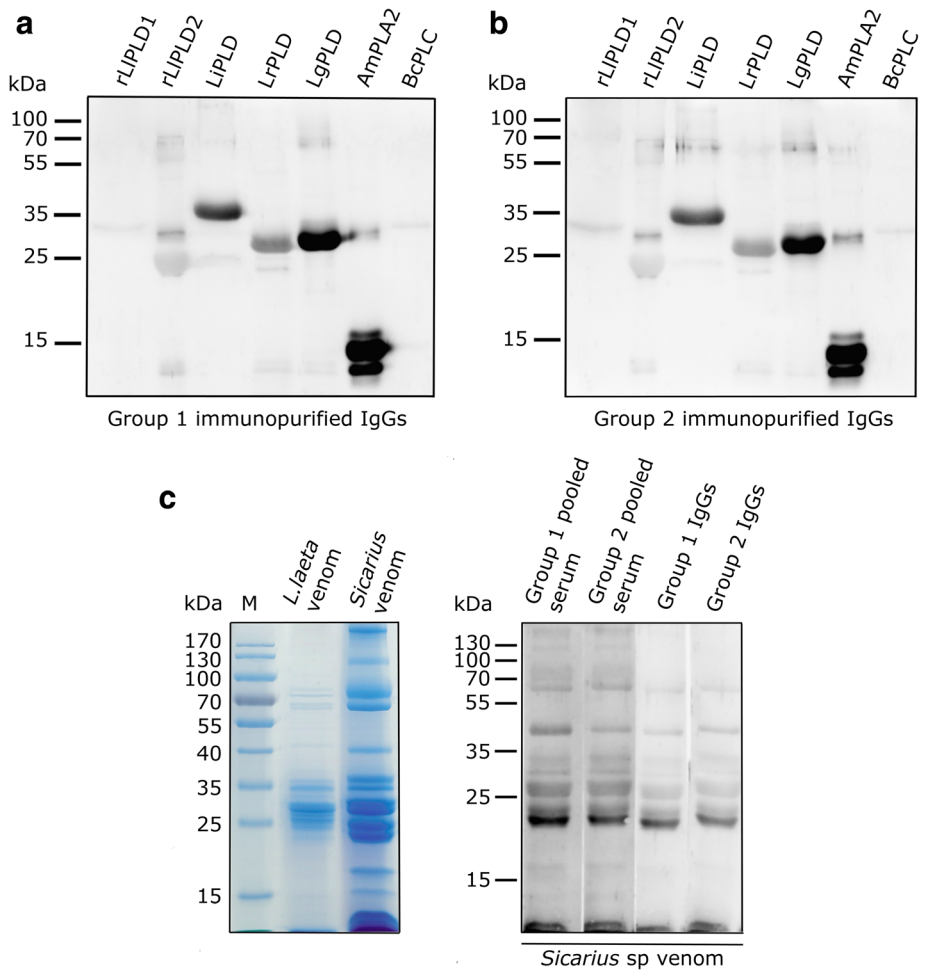

Fig. 4 Heterophilic antibodies can detect PLD from other *Loxosceles* species and the venom from *Sicarius* spiders. A quantity of 5 μg of rLIPLD1, rLIPLD2, *L. intermedia* PLD (LiPLD), *L. reclusa* PLD (LrPLD), and *L. gaucho* PLD (LgPLD), together with 5 μg PLA_2 of *Apis mellifera* venom ($AmPLA_2$), and PLC of *Bacillus cereus* (BcPLC) were separated by SDS-PAGE in 12% gel and transferred to a nitrocellulose membrane. Then, each protein was detected by incubation with immunoselected IgGs from both groups at a concentration of 1 μg/mL, followed by incubated with goat anti-human HRP-IgG antibody (1:50,000 dilution), and developed with ECL. **a** IgG purified from Group 1 sera. **b** IgG purified from Group 2 sera. **c** Immunoblot from the venom of *Sicarius*, with serum pools and purified IgG antibodies of groups 1 and 2. (Left) SDS-PAGE in 12% gel of *L. laeta* venom and *Sicarius* venom stained with Coomassie blue. (Right) Immunoblot of *Sicarius* venom detected using serum pools from Group 1 and Group 2, and IgG antibodies purified from Group 1 and Group 2 sera

with history of immunotherapy. In addition, the serum samples from loxoscelism patient group used in our study were taken from patients who received no antivenom therapy, since Chilean guidelines for loxoscelism treatment does not suggest the use of antivenom therapy [2]. Thus, the detection of *Loxosceles* venom observed in this group was as a consequence of the presence of anti *L. laeta* venom antibodies produced by themselves and not the presence of antibodies from antivenom treatment, which could lead to production of HAAAs.

Certain future considerations and cautions should be taken about *Loxosceles* antivenom immunotherapy, since it involves the use of an anti-arachnid serum produced in horses [36], which could lead to the production of human anti-animal antibodies. In this regard, it has been documented the presence of anti-horse IgG antibodies in healthy volunteers without treatment with a horse antivenom used for the treatment of snakebites [37]. The presence and specificity of IgG antibodies in patients with loxoscelism undergoing serotherapy has been previously studied [23], showing that only results from four patients out of twenty that underwent serotherapy were able to detect the *L. gaucho* venom protein component of ~ 35 kDa by immunoblot. The authors indicate that the low number of patients able to recognize the venom was due to an inhibitory effect that sequesters the circulating immunogenic material. The authors also evaluated the sera through ELISA, in which the highest recognition titer of the venom was 1:640 and the lowest was 1:80 [23]. In our study, the mean titer for loxoscelism