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Molecular identification of wolf spiders (Araneae: Lycosidae) by multiplex polymerase chain reaction

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Abstract

Ecological field studies are often hindered by the difficulty of identifying certain taxonomic groups. For example, no identification keys exist for immature Australian wolf spiders, which are important predators of insect pests. This makes it difficult to identify the majority of specimens collected in the field. We used multiplex polymerase chain reaction for the molecular identification of seven species of wolf spiders that occur commonly in *Brassica* crops in South Australia—*Hogna crispipes*, *Hogna kuyani*, *Lycosa godeffroyi*, *Trochosa expolita*, *Venator spenceri*, *Venatrix pseudospeciosa*, and a new species in a new genus ('Species A'). Species-specific primer pairs were designed according to variations in the cytochrome oxidase subunit I gene sequences among of these spider species. Diagnostic DNA fragments for each of the target species allowed species identification. This method proved to be a powerful tool for the identification of this group of arthropods that is difficult to identify based on morphology.

Keywords: *Hogna crispipes*; *Hogna kuyani*; *Lycosa godeffroyi*; *Trochosa expolita*; *Venator spenceri*; *Venatrix pseudospeciosa*; Wolf spider; Multiplex polymerase chain reaction; Molecular identification; COI

1. Introduction

To determine the role and effectiveness of predators in the control of insect pests, detailed understanding of the biology of the species involved, in particular their behaviour and ecology are essential. Accurate species identification is crucial to achieve this aim. Traditionally, identification of species is based on morphological characteristics, but morphological keys are often useful only for a particular life stage or gender, and many species cannot be identified reliably as juveniles. Wolf spiders (Araneae: Lycosidae) are important predators of insect pests in vegetable crops (Hummel et al., 2002). However, species in this family are difficult to identify based on morphological characters alone, especially in the immature stages. Juveniles collected from the field must generally be reared to the adult stage to allow accurate species identification. This process is time consuming and not always successful. Therefore it is essential to develop a quick and reliable method for identification of this important group of predators.

Multiplex polymerase chain reaction (PCR) simultaneously amplifies several fragments in a single reaction. Under certain conditions, several species can be identified using a single PCR followed by an electrophoretic separation of amplified DNA fragments. So far multiplex PCR has been described for the simultaneous detection of bacterial (Bej et al., 1990; Song et al., 2005; Way et al., 1993), mycobacterial (Bhattacharya et al., 2003), viral (Karlsen et al., 1996) and fungal (Amicucci et al., 2000) pathogens, as well as plankton (Hare et al., 2000), mites (Kumar

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et al., 1999), insects (Dang et al., 2005; Hinomoto et al., 2004; Kengne et al., 2001), and spiders (Greenstone et al., 2005).

Spiders belong to the most abundant group of predators in agricultural systems, but have received much less attention than other insect predators (Whitehouse and Lawrence, 2001). A wide diversity of spiders lives in arable fields, of which wolf spiders are one of the most abundant families (Alford, 2003). For example, a British wheat field showed densities of up to 76 individuals/m^2 during summer (Workman, 1978). With this numerical dominance, they have the potential to consume large numbers of prey. For example, they are one of the most important predators of cereal aphids, *Rhopalosiphum padi* in Europe (Mansour et al., 1992; Nyffeler and Benz, 1982). In a *Festuca-Andropogon* old field system in the United States, 21.1% of the total mortality of herbivorous insects is due to predatory pressure of wolf spiders of the genera *Hogna* and *Rabidosa* (van Hook, 1971). Studies on spiders in Australian agroecosystems are scarce, so it is difficult to evaluate the abundance of lycosids relative to other spider families. However, spider families that dominate in Northern Hemisphere studies such as Linyphiidae (Sunderland and Samu, 2000) play only a minor role in the Australian fauna (Raven et al., 2002). In Australia, lycosids appear to aggregate in certain agroecosystems (Pearce and Zalucki, 2006), and were found to be predators of *Plutella xylostella* on a vegetable farm at Virginia, South Australia (Ma et al., 2005). In Australian cotton field, wolf spiders are a dominant epigaeic predator, whilst in higher strata of the vegetation Oxyopidae are the most abundant spider family (M.A.E. Whitehouse, personal communication).

Wolf spiders constitute the fourth largest spider family, with ca. 2300 species described in 103 genera (Platnick, 2006). Their adult body size ranges from 1 to 30 mm. They pursue an array of different prey capture strategies, from permanently vagrant hunters to permanently burrowing species, and some genera are known to build permanent sheet-webs (Murphy et al., 2006). The life cycle of wolf spiders, in particular in regions with temperate climates, is generally well synchronised with the season. However, phenology varies among species (Framenau and Elgar, 2005; Schaefer, 1976). Wolf spiders also differ in their diurnal activity patterns, which means that they only forage on insects, which are active at the same time during the day (Marshall et al., 2002). Wolf spiders may also show very specific microhabitat preferences and may be susceptible to changes in habitat structure (Jõgar et al., 2004; Marshall and Rypstra, 1999). This ecological diversity may make them suitable for control of a wide variety of insect pests. However, it also means that it is vital to be able to recognise single species to evaluate and support their role in crop management practices.

Family level identification of wolf spiders is easy due to a number of unique characters, such as the eye arrangement, the lack of a retrolateral tibial apophysis on the male pedipalp, and the unique behaviour of females that carry their egg sacs attached to the spinnerets and subsequently their young on the dorsal surface of the abdomen (Dondale, 1986; Griswold, 1993). In contrast, generic and species level identification is impossible for the non-specialist, as currently no generic key exists in Australia and only one key is available that allows species level identification within a common genus, *Venatrix* (Framenau, 2006a; Framenau and Vink, 2001), in addition to reviews of some smaller, more cryptic genera (e.g., Framenau, 2006b,c; Framenau and Yoo, 2006). Species identification of spiders generally requires the examination of male and female genitalia. Hence, the morphologically conservative wolf spiders are impossible to identify accurately as juveniles.

In this paper we describe a reliable and efficient method to identify guilds of predators that are collected in field studies. Firstly we developed DNA markers that identify seven species of wolf spiders that commonly occur in *Brassica* crops in the Adelaide Region of South Australia. Then we demonstrated that multiplex PCR can be used to identify these species in a single reaction. This approach can be used in similar situations where groups that are difficult to identify are a prominent part of the biota and also for biological control strategies.

2. Materials and methods

2.1. Spider collection

Wolf spiders were collected from Pitchford's broccoli farm at Currency Creek, South Australia at night by using a head lamp (Wallace, 1937) about 1 h after sunset, when a large number of species appeared to be active. In wolf spiders, light is reflected by the tapeta, a light-reflecting layer of cells in the eyes, and the spider's presence is indicated by a bluish or greenish sparkle (Vink, 2002). Spiders were placed in 5 ml vials and transferred to the laboratory. Representative adult wolf spiders were identified to species level and a few immature spiders were maintained at 25 °C and provided with moisture and food (*Ephestia* larva) to rear them to maturity for identification.

2.2. DNA extraction

For DNA extraction from wolf spiders, samples of 2 or 3 legs were removed with clean forceps and the rest of the body was kept at −20 °C as voucher specimen. DNA from specimens representing sac spiders (Gnaphosidae), another spider family commonly encountered at the study site, and four *Brassica* crop pests (*P. xylostella*, *Pieris rapae*, *Myzus persicae* and *Brevicoryne brassicae*) as possible prey of wolf spiders was extracted in order to test the specificity of the DNA primers.

To extract purified DNA from spiders a technique was adapted from (Boom et al., 1990) and (Höss and Pääbo, 1993).