To Ray Mannin
with appreciation
for all your help!
Christoph

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# Morphological and molecular differentiation between three allopatric populations of the littoral crab *Pachygrapsus transversus* (Gibbes, 1850) (Brachyura: Grapsidae)

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The littoral grapsid crab species *Pachygrapsus transversus* (Gibbes, 1850) has a wide distribution ranging from the eastern Pacific to the eastern Atlantic and Mediterranean. The American continent and the Atlantic Ocean are believed to act as barriers for dispersal of planktotrophic larvae. The crab populations studied, from the eastern Pacific (Panama), the western Atlantic (Caribbean), and the eastern Atlantic (Spain) were, therefore, assumed to be genetically isolated. Remarkable differences in DNA sequence of the 16S mt-DNA gene as well as in larval and adult morphometry and coloration were found between these populations. A closer genetic similarity between the amphi-Atlantic populations in comparison to the Pacific animals, suggests the occurrence of occasional gene flow across the Atlantic Ocean.

KEYWORDS: DNA sequence, morphometry, allopatric, population, biogeography, dispersal, gene flow.

## Introduction

The dispersal of marine intertidal invertebrates is limited by either major continents or ocean basins. Even planktotrophic larvae with an average duration in the marine plankton of approximately 2–6 weeks are, in most cases, unable to overcome these biogeographical barriers (Thorson, 1961; Scheltema, 1986). Species with a wide distribution and occurring on both sides of such barriers are therefore especially well suited for studying differentiation among allopatric populations and genetic isolation.

The grapsid crab *Pachygrapsus transversus* occurs along subtropical and tropical rocky shores on both sides of the Atlantic and the eastern Pacific (Rathbun, 1918; d'Udekem d'Acoz, 1992; Flores, 1996) including the Galápagos Islands (Garth, 1946), the West Indies (Chace and Hobbs, 1969), several East Atlantic islands (Türkay, 1982) and parts of the Mediterranean Sea (e.g. Holthuis and Gottlieb,

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1958; Kinzelbach, 1964). In this analysis we studied crabs from three geographical regions which are separated from each other by either the American continent or the Atlantic Ocean. We therefore assumed that they are genetically isolated. This study demonstrates the existence of three distinct populations of *P. transversus* which can be distinguished by larval and adult morphometry, coloration, and DNA sequence.

## Materials and methods

Crabs were collected in 1994 and 1996, most during a visit to the Smithsonian Tropical Research Institute (Panama) in the spring of 1996. Eastern Pacific crabs were from Naos, Farfán Beach and Taboga Island (all Panama). In the western Atlantic, crabs were collected from María Chiquita (Panama), Florida, Jamaica, Antigua, Puerto Rico, Grenada, and Curaçao. Eastern Atlantic specimens were from Cádiz (Spain). DNA was sequenced from crabs collected at Naos (eastern Pacific), María Chiquita and Antigua (western Atlantic), and Cádiz (eastern Atlantic). Crab tissue for DNA sequencing was preserved in 75% ethanol. DNA was obtained from the muscle tissue of walking legs using a phenol-chloroform extraction. Selective amplification of a fragment from the mitochondrial large subunit (16S) r-RNA gene was carried out with polymerase-chain-reaction (PCR) using the primers 16sar (5′-CGCCTGTTTATCAAAAACAT-3′) and 16sbr (5′-CCGGTCTGAACTCAGATCACGT-3′) (Palumbi *et al.*, 1991) and a temperature profile of 94°C/50 55°C/72°C. Asymmetric PCR amplification yielded single stranded DNA (Gyllenstein and Erlich, 1988) which was marked with radioactive sulphur, sequenced by dideoxy sequencing (Sanger *et al.*, 1977) and separated on 6% polyacrylamide gels. Sequence data were read from autoradiograms and aligned by hand with the multisequence editing program ESEE (Cabot and Beckenbach, 1989).

Morphometric analyses were carried out by measuring at least 20 adult crabs from each geographical region (total of 77), consisting of approximately equal numbers of males and females. First zoea larvae were obtained in the laboratory from ovigerous females collected at Naos and Farfán (eastern Pacific, 3 hatches), María Chiquita (western Atlantic, 1 hatch) and Cádiz (eastern Atlantic, 2 hatches). Up to 40 larvae per hatch were measured using an ocular micrometer on a binocular microscope. Crabs used for DNA sequencing and for photographs have been deposited at the Senckenberg-Museum, Frankfurt a. M. (SMF: 23717 23720).

## Results

Sequence comparison of 510 basepairs of the 16S mt-DNA gene of *Pachygrapsus transversus* revealed three different haplotypes, corresponding to the different populations. Divergence percentages between haplotypes are shown in table 1. Furthermore, a continuous segment of 16 nucleotides is presented as an example for genetic differences between the sequences (table 1). The homologous sequence of the closely related eastern Pacific species *Pachygrapsus crassipes* (Randall, 1839) from Los Angeles (California, USA) was included for additional comparison. Sequences of *P. transversus* from two Caribbean sites (Antigua and María Chiquita, Panama) were identical. The sequence from the eastern Atlantic (Cádiz, Spain) differs from the western Atlantic haplotype by five mutations. Much more pronounced differences (17 18 mutations) were found when comparing all Atlantic *P. transversus* with the East Pacific representative from Naos (Panama). The number of mutations of all *P. transversus* to *P. crassipes* was more or less constant and ranged between 36 and 40.

Table 1. Per cent divergence among haplotypes within 510 basepairs of 16S mt-DNA from three allopatric populations of *Pachygrapsus transversus* (Gibbes. 1850) and *Pachygrapsus crassipes* (Randall). An alignment of 16 basepairs of the DNA sequence shows some of the mutations between these populations and species.

| | % sequence divergence | | | |
|---|---|---|---|---|
| Site (Population) | Cádiz | Antigua | Ma. Chiquita | Naos |
| Cádiz; Spain (East Atlantic) | | | | |
| Antigua; Lesser Antilles (Caribbean) | 1.0 | | | |
| María Chiquita; Panama (Caribbean) | 1.0 | 0 | | |
| Naos; Panama (East Pacific) | 3.5 | 3.3 | 3.3 | |
| *Pachygrapsus crassipes* (East Pacific) | 7.1 | 7.1 | 7.1 | 7.8 |

| | DNA sequence fragment |
|---|---|
| Cádiz; Spain (East Atlantic) | G T G A G T A A C A G G G A G T |
| Antigua; Lesser Antilles (Caribbean) | G T G A G T A A C A G G A A G T |
| María Chiquita; Panama (Caribbean) | G T G A G T A A C A G G A A G T |
| Naos; Panama (East Pacific) | G T G A G T      G A G A A A G T |
| *Pachygrapsus crassipes* (East Pacific) | G T G A A T A A A G A A G A G T |

Comparison of larval and adult morphometry showed significant differences between the three populations of *P. transversus* (table 2). Next to differences in total size of the first zoea, we found that the ratios of dorsal versus rostral carapace spine length clearly differed between populations (table 2, figure 1). Also the dentation of the ventral margin of the cephatothorax and the antennae of the larvae showed consistent differences between populations (figure 1). Morphometric differences

Table 2. Differences in adult and larval morphometry between three allopatric populations of *Pachygrapsus transversus* (Gibbes, 1850). Values represent means, standard deviations, and sample sizes. Adult samples were tested for normal distribution and compared by means of a 1-Factor-ANOVA and Fisher PLSD Post hoc tests. Numbers in parentheses following the significance levels indicate: (1) Caribbean *P. transversus* significantly different from other two populations; (2) East Pacific *P. transversus* significantly different from other two populations. Larvae were from few hatches; the data are thus not independent and cannot be used for statistical analyses. Abbreviations: cl: carapace length; cw: maximal carapace width; pcw: width at posterior carapace; 4ppl: total length of 4th pereiopod; 4ml/mw: merus length / merus width of 4th pereiopod.

| | | East Atlantic | Caribbean | East Pacific | F | p |
|---|---|---|---|---|---|---|
| **Adults** | | | | | | |
| cl/cw | males | 0.8 ± 0.01 (17) | 0.77 ± 0.01 (13) | 0.81 ± 0.01 (11) | 44.09 | 0.001 (1) |
| | females | 0.77 ± 0.01 (17) | 0.75 ± 0.01 (8) | 0.77 ± 0.01 (11) | 12.59 | 0.001 (1) |
| pcw/cw | | 0.79 ± 0.02 (33) | 0.79 ± 0.03 (21) | 0.81 ± 0.02 (22) | 11.3 | 0.001 (2) |
| 4ppl/cw | males | 1.28 ± 0.04 (17) | 1.31 ± 0.08 (13) | 1.41 ± 0.03 (11) | 18.05 | 0.001 (2) |
| | females | 1.25 ± 0.04 (15) | 1.24 ± 0.08 (8) | 1.3 ± 0.05 (11) | 3.42 | 0.05 (2) |
| 4ml/mw | | 1.88 ± 0.1 (31) | 2.0 ± 0.09 (21) | 1.92 ± 0.1 (22) | 8.43 | 0.001 (1) |
| **Larvae** | | | | | | |
| total length [mm] | | 0.75 ± 0.02 (60) | 0.62 ± 0.03 (20) | 0.64 ± 0.03 (47) | | |
| dorsal/rostral spine | | 1.07 ± 0.05 (60) | 0.94 ± 0.03 (20) | 0.85 ± 0.11 (47) | | |

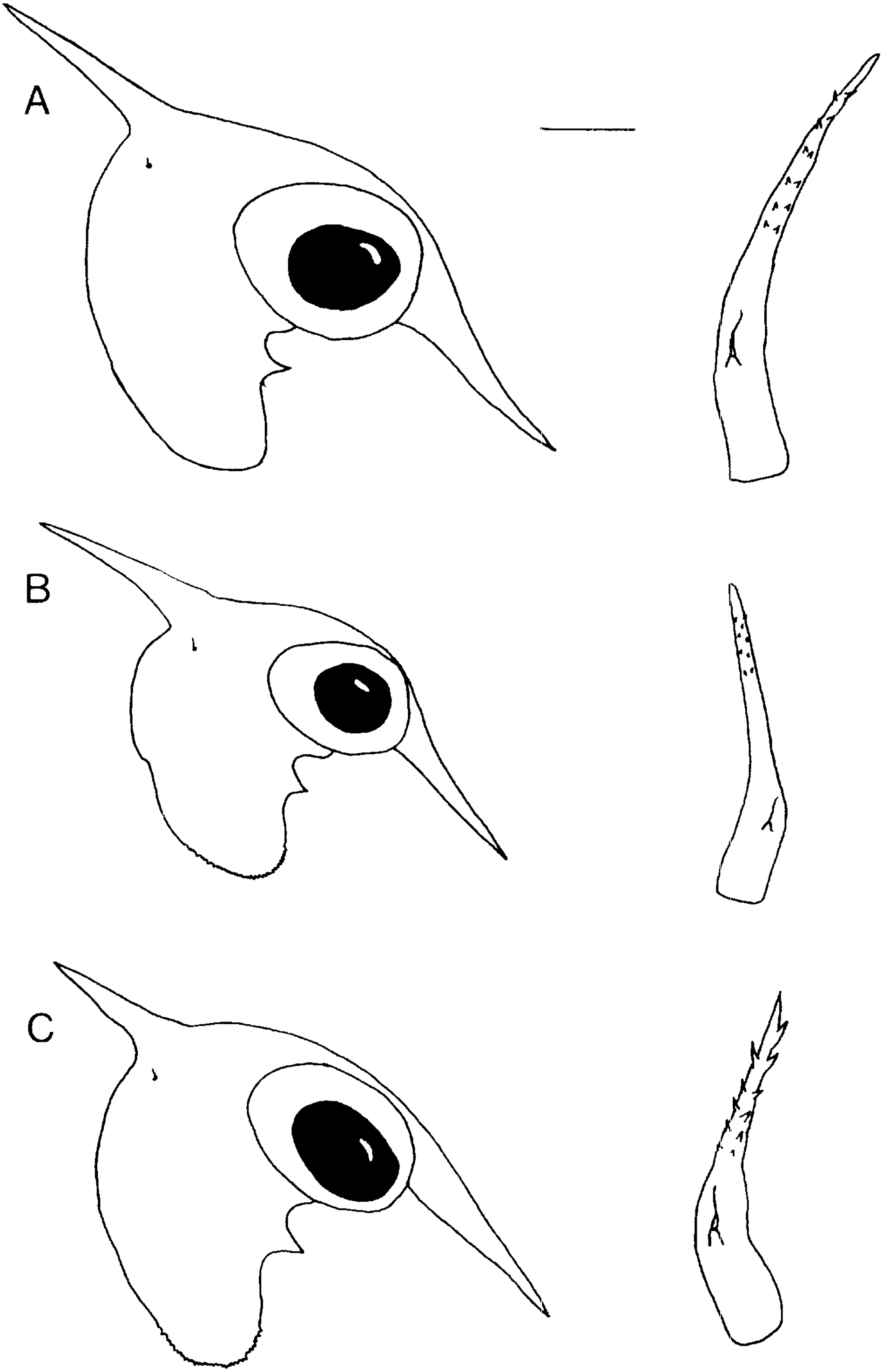

FIG. 1. Schematic representations of zoea I lateral view and antenna from three allopatric populations of *Pachygrapsus transversus* (Gibbes, 1850): (A) Cádiz, East Atlantic; (B).

between adult specimens of the three populations are summarized in table 2. Significant differences were found in relative carapace length (see also Flores, 1996) and length of the fourth pereiopod (table 2) not only between populations, but also between sexes. As an additional separating character we found differences in the colour pattern of the chela. The outer face of the palm is consistently dark around the dactylus articulation in the Atlantic crabs, while light cream in the Pacific ones (figure 2).

## Discussion

The existence of cryptic and sibling species among decapod Crustacea shows that closely related species are often difficult to distinguish by morphological characters (Knowlton, 1986; Felder and Staton, 1994). If this holds true for the adults, it becomes even more evident when trying to tell apart larvae of closely related species (e.g. Clark, 1983). Furthermore, very little is known about the degree of intraspecific variability and how far it may affect ability to differentiate similar species (Jensen *et al.*, 1992). In the case of *Carcinus maenas* (L.) and *C. aestuarii Nardo* (as *C. mediterraneus*), Rice and Ingle (1975) only found differences in the ratio of dorsal versus rostral spine between these possibly distinct species. Jensen *et al.*, (1992) observed that the zoea I of two species of *Paralithodes* could be distinguished most reliably by the proportion of rostrum to carapace length, when the overlap in telson spine counts was greatest. In this study we found marked differences in larval morphometry on an intraspecific level between three allopatric populations of *Pachygrapsus transversus* (table 2, figure 1). The use of larval size for taxonomic separation is problematic, because it seems to be geographically variable, possibly depending on water temperatures (Shirley *et al.*, 1987). Also, measurements can be easily affected by the observer's technique. The total size of our zoea I of the Caribbean (0.62 ± 0.03 mm) agrees with the size given by Ingle (1987) of 0.6 to 0.7 mm, but not with Lebour's (1944) 0.9 mm, both measured for *P. transversus* larvae from Bermuda. Cuesta and Rodríguez (1994) measured a total size of 0.71 ± 0.02 mm for the zoea I from Cádiz, which is slightly smaller than our measurements of two hatches from the same site (0.75 ± 0.02 mm, table 2). Overall, the East Atlantic larvae from the warm temperate waters of Cádiz are clearly larger than the tropical Caribbean and Pacific populations. Other data from larval morphometry suggesting differences between the three populations are the ratios of dorsal versus rostral spines of the zoea I (figure 1, table 2).

Differentiation between three allopatric populations of *Pachygrapsus transversus* was confirmed by significant differences in adult morphometry (table 2), different colour patterns of the adult chelae (figure 2) and DNA sequence (table 1). Differences in the coloration of the chelae were noted by Stimpson (1871) in his description of the Pacific *Pachygrapsus socius* (junior synonym of *P. transversus*). Marked differences between the three populations of *P. transversus* contrast with low variability within these populations (tables 1–2). Genetic isolation between these allopatric forms therefore seems to be confirmed.

The separation between East Pacific and West Atlantic *P. transversus* can be timed rather accurately. The closure of the Panama Land Bridge was completed about 3.1 million years ago (Keigwin, 1982), splitting the Central American marine fauna into Pacific and Caribbean elements. As a consequence, several geminate species have been recognized in different taxonomic groups (Rubinoff and Rubinoff, 1971; Vawter *et al.*, 1980; Lessios, 1981; Weinberg and Starczak, 1989; Knowlton

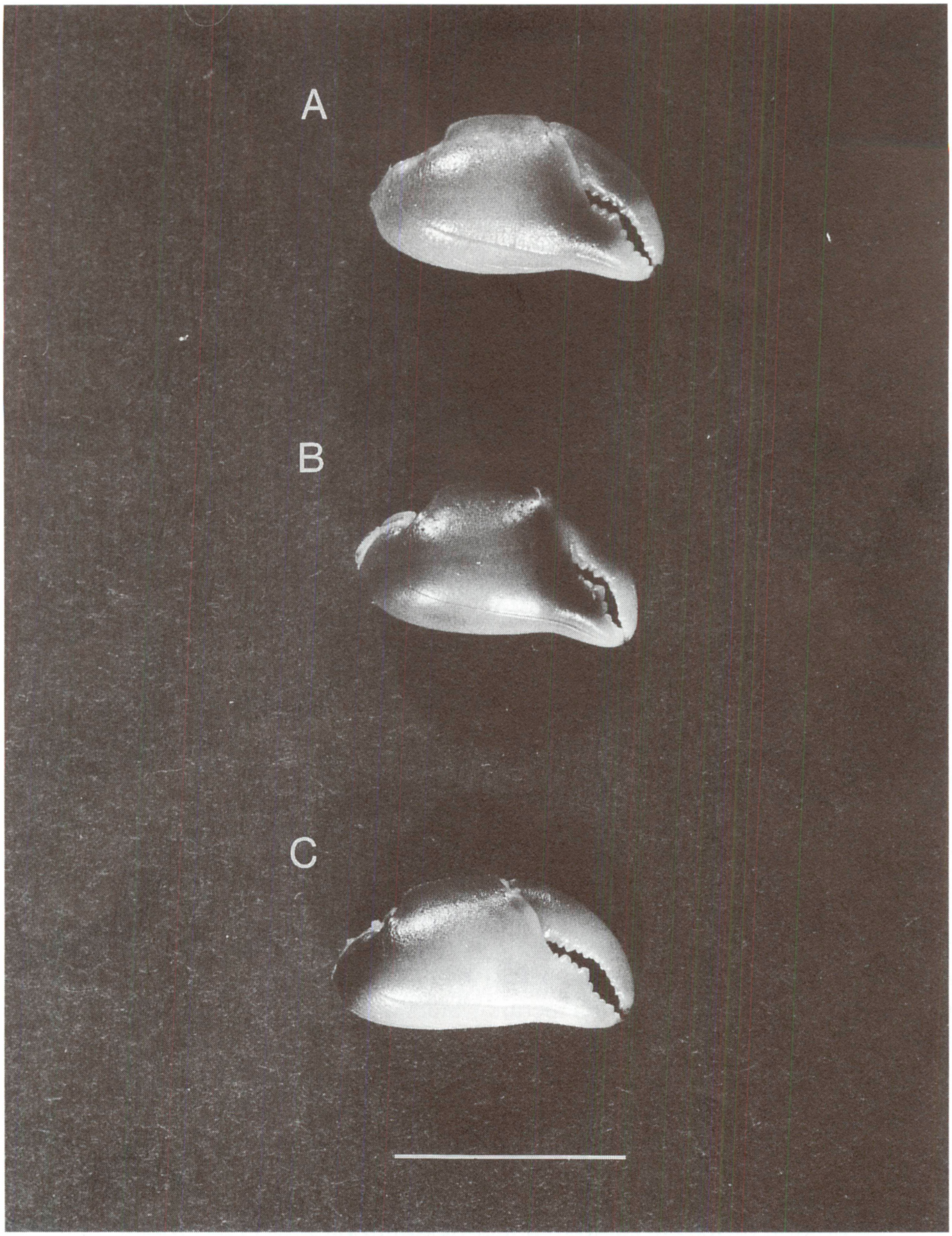

FIG. 2. Outer faces of right adult chelae showing differences in colour pattern in three allopatric populations of *Pachygrapsus transversus* (Gibbes, 1850): (A) Cádiz, East Atlantic; (B) Antigua, Caribbean; (C) Naos, East Pacific. Scale bar 1 cm.

*et al.*, 1993). The interruption of gene flow across Central America and the restriction of *P. transversus* to subtropical and tropical waters rules out any potential genetic exchange between the Atlantic and Pacific populations. Only after the construction of the Panama Canal, a secondary contact across the Panama Isthmus might be possible. The genetic identity found between the 16S mt-DNA gene of *P. transversus* from the Lesser Antillean island Antigua and the one from the Panamanian

Caribbean coast (next to the opening of the Canal) shows that no Pacific influence is currently noticeable in the Caribbean population of this crab species.

It is not possible to relate genetic isolation across the Atlantic Ocean to a single geomorphological event. Higher current velocities and a smaller size of the Atlantic Basin during the Early Tertiary allowed a passively drifting larva to cross the Atlantic in 2 to 4 weeks (Scheltema, 1986). Today it would take from 9 to 28 weeks (Thorson, 1961; Scheltema, 1986), well above the time brachyuran larvae are believed to spend in the marine plankton. Hines (1986) calculated an average of 31 ± 7 days of zoeal period for the Grapsidae. The Atlantic thus seems to act as a filter for planktonic dispersal. Assuming that this filter represents an insuperable obstacle, isolation between the East and West Atlantic *P. transversus* should have occurred sometime during the Miocene, around 10 million years ago (see table 1 in Scheltema, 1986). Our results show that differences are more pronounced between the two American populations of *P. transversus* than between the two Atlantic populations (table 1, figure 2). Complete isolation across the Atlantic must therefore have occurred later than presumed or alternatively, an occasional dispersal across the Atlantic Ocean is taking place. Rosenblatt and Waples (1986) interpret similar findings of less genetic distance in shore fishes across the Pacific Barrier than across the Panama land bridge as ongoing gene flow. Dispersal capabilities should increase with longer duration of the pelagic phase. Schlotterbeck (1976) raised *Pachygrapsus crassipes* to a fifth zoeal stage at 16°C, before the larvae died after 95 days. Wilson and Gore (1980) claim a minimum time of 60 days for completion of larval development of *Plagusia depressa* (Fabricius, 1775). It is therefore likely that some of the grapsid crab species have a longer larval life than often believed and thus should have a greater potential for larval dispersal (see also Manning and Chace, 1990). On the other hand, Hines (1986) did not find a significant relationship between extent of the range and duration of the larval dispersal period (see also Palumbi, 1995). When discussing the possibility of dispersal across large ocean basins, the role of oceanic islands (e.g. Ascension, St. Helena, St. Paul in the Atlantic) as intermediate stations should also be taken into account. An additional form of dispersal is adult rafting on floating objects. Referring to the grapsid genera *Plagusia*, *Planes*, and *Pachygrapsus*, Garth (1966) noted that they are 'habitually transported on drifting logs or on sea turtles as adults'. Accidental transport on fast travelling ships is a phenomenon of 'modern times'. The biogeographic relevance of this mode of dispersal has only recently been studied (Carlton, 1985), while ships have already travelled regularly between the Old World and American waters during the times of colonialism and slave trading.

This study strongly suggests differentiation among three populations of the grapsid crab *Pachygrapsus transversus*. Since differences were consistent within populations, a taxonomic separation might appear reasonable. On the other hand, we feel the need to study crabs from additional sites of this widely distributed species, before postulating new subspecies or species for entire coastlines. Morphologic and genetic differences outlined in this study should be confirmed for as many sites as possible along the ranges of these populations. Garth (1946) describes the movable finger of the chela of *P. transversus* from the Galápagos islands as shading to purple and Manning (personal communication) noted that the articulations of the limbs were of a bluish colour in specimens from the North African coast, both being coloration patterns never observed by us. Inclusion of additional specimens might therefore reveal further distinct forms, which should be taken into account before undertaking a taxonomic revision for this polymorphic species.

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