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New insights into *Plutonium*, one of the largest and least known European centipedes (Chilopoda): distribution, evolution and morphology

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Plutonium zwierleini is one of the largest scolopendromorph centipedes in Europe and is known for debates on the evolution of segmental body pattern in arthropods. However, only 24 records have so far been reported and probably only a dozen specimens still exist in collections. Based on 19 new data from professionals and amateur recorders, we confirmed that populations are present today in four disjunct areas: southern Iberian Peninsula, Sardinia, southern Italian Peninsula and Sicily. A phylogenetic analysis of the first molecular data obtained for *Plutonium* (*16S*, *COI*, *18S*, *28S*) provided strong evidence that it is closely related to *Theatops*, either sister to the latter or nested within it, with an estimated separation since the Mesozoic. By examining specimens of *Plutonium* from all the four inhabited areas and specimens of most of the species of *Theatops*, we also documented many synapomorphies uniting *Plutonium* and *Theatops* (brown-orange colour, whitish patches replacing the ocelli, a particular denticulation on the forcipules, conspicuously swollen and piercing ultimate legs) and some derived characters unique to *Plutonium*, including the blade-edged claws of the ultimate legs and the previously overlooked presence of long, dense setae on most other legs, in both sexes and in the entire species range.

ADDITIONAL KEYWORDS: body size – phylogeny – *Plutonium zwierleini* – Scolopendromorpha – *Theatops*.

INTRODUCTION

Of the European scolopendromorph centipedes, only two species have a large body size and are potentially harmful to humans. One is the well-known *Scolopendra cingulata* Latreille, 1829, which is frequently encountered in the southern regions (Simaiakis & Mylonas, 2008; Lewis, 2010) and the other is *Plutonium zwierleini* Cavanna, 1881, which is an almost neglected species even though it may reach a body length of 12 cm and, in addition to the anterior venomous forcipules, it is armed with a pair of strongly uncinated ultimate legs (Edgecombe & Bonato, 2011).

Plutonium zwierleini, the only species of the genus *Plutonium*, was discovered more than 130 years ago (Cavanna, 1881), but specimens have been found only rarely. As a result, the morphology of the species is still only partially known and its geographical distribution and ecology are very uncertain.

Since its original description, *Plutonium* has intrigued taxonomists and evolutionary biologists like no other centipede, especially because of the unusual segmental arrangement of the respiratory openings. In all other Scolopendromorpha, the spiracles are present on approximately alternating leg-bearing segments, but in *Plutonium* they are present on all leg-bearing segments from the second to the penultimate, as in the Geophilomorpha (Fusco, 2005). This unusual condition has fuelled debates on the relationship between *Plutonium* and the remaining scolopendromorphs (e.g. Schileyko & Pavlinov, 1997; Shelley, 1997) and led to speculation on the evolutionary trends in the segmental architecture of centipedes and arthropods at large (e.g. Manton, 1965; Schileyko, 1992; Minelli *et al.*, 2000). Under the now outdated hypothesis that arthropods evolved from a homonomously

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segmented annelid-like ancestor (cf. Fusco & Minelli, 2013), *Plutonium* was seen as a sort of 'evolutionary link' between the putative ancestral homonomous segmentation of geophilomorphs and the 'more advanced' heteronomous segmentation of the remaining centipedes. However, modern phylogenetic investigations concur in considering the geophilomorph condition as derived, rather than primitive, within the centipedes (e.g. Murienne, Edgecombe & Giribet, 2010). In addition, some morphological features suggest that *Plutonium* is actually a derived scolopendromorph belonging to a well-supported monophyletic subgroup, informally labelled as the 'blind clade', which includes Plutoniumidae, Cryptopidae and Scolopocryptopidae (Vahtera, Edgecombe & Giribet, 2012a). Within the 'blind clade', *Plutonium* is united in the family Plutoniumidae with the genus *Theatops*, which comprises six species distributed across a broad area in North America and a few more restricted regions in Eurasia (Shelley, 1997; Di *et al.*, 2010).

As a consequence of the central role of *Plutonium* in the debate on centipede and arthropod evolution, in the very few specimens collected in the past, several features have been examined in a search for key evolutionary traits, including integument histology (Passerini, 1883), functional anatomy of the legs (Verhoeff, 1906) and the anatomy of nervous system (Prunesco, 1970a), peristomatic structures (Edgecombe & Koch, 2008, 2009), gut (Koch, Pärschke & Edgecombe, 2009) and reproductive system (Prunesco, 1970b, 1997). Until now, however, the external morphology of *P. zwierleini* has not been investigated in full. Additionally, a shortage of specimens in zoological collections and repeatedly unsuccessful dedicated field collection campaigns have impeded an evaluation of intraspecific morphological variation and the obtainment of molecular data to assess the phylogenetic position of the species.

To contribute to a better understanding of the geographical distribution, evolutionary origin and morphology of this species, we have integrated different approaches and data sources, including (1) a call to other professionals and amateur recorders for new records, (2) the phylogenetic analysis of molecular data obtained for *P. zwierleini* for the first time and (3) a direct comparison of specimens from all separate areas inhabited by the species. In the present paper, we provide (1) a revised and expanded view of the geographical distribution of *Plutonium*, (2) a molecular estimation of its phylogenetic position and divergence time from its closest relatives and (3) a detailed illustration of its unique morphological features, especially in comparison with its closest extant relatives.

MATERIAL AND METHODS

GEOGRAPHICAL RECORDS

As far as possible, we retrieved all published records of *P. zwierleini* by evaluating all citations of *Plutonium* in the primary taxonomic and faunistic literature (85 publications, to the best of our knowledge).

We also conducted a census of the specimens available in zoological collections by referring to the available catalogues of all major European museums hosting myriapod collections, by contacting the curators of 61 museums and institutions and by sending a request for information to >150 members of a myriapodology electronic mailing list.

To gather new records, we requested information from 57 naturalists' and speleologists' groups and associations that are active in the regions from which *P. zwierleini* had been recorded. New records were validated only when documented by voucher specimens and/or photographs taken in the field. We thought that such an approach would be suitable and effective for our target species, because (1) occasional encounters are expected to be noticed by people because of the large size and threatening appearance of these animals, (2) most of the few records obtained in the past came from occasional occurrences in urban areas, even inside buildings, or in caves (e.g. Zapparoli, 2009) and (3) identification of the species could be confirmed unambiguously based on photos taken with cameras commonly in use because of some conspicuous diagnostic features, including body size, colour and shape of the posterior end of the body.

The records were mapped by combining published indications with information retrieved from labels of preserved specimens and by word of mouth.

MOLECULAR PHYLOGENETIC ANALYSIS

DNA extraction and sequencing

Total DNA was extracted from a few legs of a specimen of *P. zwierleini* preserved in 100% ethanol (from Sardinia, Grotta di Istirzili, near Baunei, 12 May 2013, C. Onnis leg., coll. Zapparoli), using a DNeasy Blood and Tissue kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. We amplified four genes that are commonly employed in centipede phylogenetics (e.g. Murienne *et al.*, 2010): the mitochondrial genes for 16S rRNA and cytochrome *c* oxidase subunit I (*COI*) as well as the nuclear genes for 18S and 28S rRNA. *16S* was amplified using the primer pair 16Sa/16Sb (Edgecombe, Colgan & Sharkey, 2006), *COI* was amplified using LCO1490/HCOout (Folmer *et al.*, 1994; Carpenter & Wheeler, 1999), *18S* was amplified in three overlapping fragments using 1F/18SnewPluRev, 3F/18Sbi and 18SIIInewPluFor/9R (Giribet *et al.*, 1996, Whiting *et al.*, 1997) and *28S* was amplified in two overlapping fragments using 28Sa⁄28Srd5b and 28S4.8a⁄28Srd7b1 (Whiting *et al.*, 1997; Schwendinger & Giribet, 2005). The primers 18SnewPluRev (CGCAACAACTTTAATATACGCT) and 18SIIInewPluFor (CTCAACACGGGAAAACTCAC) were designed specifically for *P. zwierleini 18S*.

PCRs were performed in 20 µL reactions containing 4 µL of 5× Flexi buffer, 0.4 µL of 10 mM dNTPs, 1.2 µL of 25 mM $MgCl_2$, 0.5 µL of 100% DMSO, 0.5 µL of 10 µM primers, 0.1 µL of 5 U/µL GoTaq G2 Flexi DNA Polymerase (Promega, Madison, WI, USA), 1 µL of template DNA and purified water. The reaction was carried out as follows: first step at 95 °C for 5 min; then 27–35 cycles consisting of 1 min at 94 °C, 1 min at 40–56 °C and 1 min 30 s at 72 °C; a final step at 72 °C for 7 min. To avoid undesired fragments obtained when amplifying *COI*, the PCR products were cloned into the plasmid vector pCRII (Invitrogen, Carlsbad, USA), transforming NovaBlue Singles Competent Cells (Novagen, Madison, WI, USA), and several clones were screened for each fragment obtained. Samples were purified using a MinElute PCR purification kit (Qiagen) and then directly sequenced on both strands with the same primer sets as used for amplification, by means of an ABI 3130 XL automatic capillary sequencer (Applied Biosystems, Branchburg, NJ, USA; service provided by BMR Genomics, Padova, Italy). Sequences were deposited in GenBank (Table 1).

Taxon sampling

To evaluate the phylogenetic position of *Plutonium* within the so-called 'blind clade' of scolopendromorph centipedes (Vahtera *et al.*, 2012a), the sequences of the four genes obtained for *P. zwierleini* were analysed together with already available homologous sequences of another 15 species belonging to Plutoniumidae, Cryptopidae and Scolopocryptopidae (Table 1). Species were chosen to be representative of the taxonomic disparity of the clade and, as far as possible, from among those for which comparably long sequences were available for all four genes (Murienne, Edgecombe & Giribet, 2010; Vahtera *et al.*, 2012a; Vahtera, Edgecombe & Giribet, 2013). Sequences from multiple specimens, whenever available, were included for species of *Theatops*, which was retrieved as the closest genus to *Plutonium* in previous morphological analyses (Vahtera *et al.*, 2012a; Vahtera Edgecombe & Giribet, 2012b, 2013). Additionally, sequences referred to *Lithobius forficatus* (Linnaeus, 1758) (Lithobiomorpha) were included as an outgroup.

Sequence alignment

The *16S* sequences were aligned using RNAsalsa (Stocsits *et al.*, 2009) with default parameters and constrained with the maximum expected accuracy structure calculated by RNAalifold (Bernhart *et al.*, 2008), which is included in the Vienna RNA package v.2.1.9 (Lorenz *et al.*, 2011). Randomly similar sections were identified in the alignment by Aliscore v.2.0 (Misof & Misof, 2009), setting default window size and the –N option, and then trimmed. The sequences of the other genes were aligned with MAFFT v.7.221 (Katoh & Standley, 2013) using the L-INS-i algorithm (Katoh *et al.*, 2005) at default parameters. Poorly aligned regions and terminal gaps were removed (Table 2). The *28S* alignment was processed with both Aliscore and ZORRO (Wu, Chatterji & Eisen, 2012), by removing all positions with posterior probability < 0.4. Pairwise *p*-distances of the *COI* sequences were calculated using MEGA 6 (Tamura *et al.*, 2010), treating gaps with partial deletion and estimating standard errors by 500 bootstrap pseudoreplicates.

Phylogenetic analyses

Analyses were performed by maximum parsimony (MP), maximum likelihood (ML) and Bayesian inference (BI), both on the single genes and on their combination. All trees were rooted on the basis of the well-established monophyly of the scolopendromorphs (e.g. Murienne *et al.*, 2010) and were visualized with Fig Tree v.1.4.2.

MP analyses were performed with TNT (Goloboff, Farris & Nixon, 2008) using an heuristic traditional search with 50 randomized stepwise addition replicates, followed each by tree-bisection-reconnection branch swapping, retaining up to 20 trees per replicate. The strict consensus was generated when multiple equally parsimonious trees were found. Node support was assessed with 1000 bootstrap replicates. Search of the MP tree was achieved on equal weighting scheme for both character and transition matrix and after removing all alignment columns containing gaps and ambiguous bases.

ML analyses were performed with PhyML v.3.2 (Guindon *et al.*, 2010) after selecting the best fitting models among 54 general time reversible models, according to both AIC and BIC criteria, using jModelTest v.2.1.6 (Posada, 2008). The selected models were TVM+I+G for *16S*, TIM+I+G for *COI*, TrNef+I+G for *18S* and GTR+G+I for *28S*. The model parameters were estimated by maximizing the likelihood function. For each gene, an a posteriori parametric bootstrap with the ML parameter values was carried out in p4 (Foster, 2004) with 5000 replicates, to test for the fit of the sequences to the null hypothesis of stationarity (uniform base composition as in the model used for the inference). Node support was assessed with 500 bootstrap replicates, and convergence of node frequencies retrieved from bootstrap replicates was checked in

Table 1. Species and DNA sequences included in the phylogenetic analysis

Species	Label	Country: locality	Specimen code	Accession number			
				COI	*16S*	*18S*	*28S*
Cryptops hortensis (Donovan, 1810)	Cryp_horte	United Kingdom	MCZ IZ130582	JX422662	JX422684	JX422708	JX422597
Cryptops lamprethus Chamberlin, 1920	Cryp_lampr	New Zealand	MCZ DNA103950-IZ130584	JX422663	JX422685	JX422709	JX422583 (*28Sb*) JX422598 (*28Sc*)
Cryptops sarasini Ribaut, 1923	Cryp_saras	New Caledonia	MCZ DNA103948-IZ130605	JX422664	JX422687	JX422711	JX422585 (*28Sb*) JX422600 (*28Sc*)
Cryptops weberi (Pocock, 1891)	Cryp_weber	Indonesia	MCZ DNA102459-IZ130609	HQ402551	KF676464	HQ402518	HQ402535
Newportia divergens Chamberlin, 1922	Newp_diver	Guatemala	MCZ DNA104725-IZ130736	JX422668	JX422691	JX422714	–
Newportia ernsti Pocock, 1891	Newp_ernst	Dominican Republic	MCZ DNA105917-IZ130773	JX422669	JX422692	JX422715	–
Newportia longitarsis (Newport, 1845)	Newp_longi	Colombia	MCZ DNA104706-IZ130774	JX422670	JX422693	JX422716	JX422588 (*28Sb*) JX422604 (*28Sc*)
Newportia monticola Pocock, 1890	Newp_monti	Costa Rica	MCZ DNA103963-IZ130777	KF676507	HQ402497	HQ402514	KF676360 (*28Sb*) HQ402531 (*28Sc*)
Newportia quadrimeropus (Shelley & Mercurio, 2005)	Newp_quadr	Mexico	MCZ DNA104639-IZ130826	HQ402546	HQ402494	HQ402511	–
Plutonium zwierleini Cavanna, 1881	Plut_zwier	Italy: near Baunei	PD-S-0221	LN890292	LN890289	LN890290	LN890291
Scolopocryptops macrodon (Kraepelin, 1903)	Scol_macro	French Guiana	MCZ DNA105858-IZ130814	JX422675	JX422699	JX422721	JX422607
Scolopocryptops melanostoma Newport, 1845	Scol_melan	Costa Rica	MCZ DNA104714-IZ130816	JX422676	JX422700	JX422722	KF676362 (*28Sb*) JX422608 (*28Sc*)
Scolopocryptops miersii Newport, 1844	Scol_miers	French Guiana	MCZ DNA104699 [1]AMNH IZC00146511 [2]AMNH LP3868	[1]HQ402545	[2]JX422698	[1]HQ402510	[1]HQ402528
Scolopocryptops nigridius McNeill, 1887	Scol_nigri	North Carolina	MCZ DNA105919-IZ130806	JX422680	JX422704	JX422726	JX422594 (*28Sb*) JX422612 (*28Sc*)
Theatops erythrocephalus C.L. Koch, 1847	Thea_eryt1	Spain: near Barcelona	MCZ DNA103996	JX422665	JX422689	AF000776	–
	Thea_eryt2	Portugal: Sierra do Gerés	MCZ DNA104805	HM453313	HM453222	–	HM453279
Theatops posticus (Say, 1821)	Thea_post1	North Carolina: near Durham	MCZ DNA100806-IZ131448	AY288746	AY288727	AY288695	HM453280
	Thea_post2	Florida: Torreya State Park	MCZ DNA105630-IZ130612	JX422666	JX422688	JX422712	–
Outgroup: *Lithobius* cf. *forficatus* (Linnaeus, 1758)	Lith_forfi	[1] Michigan [2] Germany [3] unknown [4] Austria	[3]MCZ DNA102105-IZ131533	[1]AF309492	[2]AF373608	[3]EU024571	[4]EF199984

Table 2. Number and length of DNA sequences employed in the phylogenetic analyses

Gene	Number of sequences	Original length (bp)		Length after trimming (bp)
		Min	Max	
16S	19	416	539	295
COI	19	656	1533	715
18S	18	1807	1864	1843
28S	14	868	3913	733

RAxML v.8 (Stamatakis, 2006, 2014) with the –I option. Alternative hypotheses on the position of *P. zwierleini* were compared with a Shimodaira–Hasegawa test implemented in Consel (Shimodaira, 2002). The same test was also performed after gap removal, and the effect of indels on tree inference was evaluated using the pipeline proposed by Grievink, Penny & Holland (2013) and the same models reported above.

BI analyses were performed with BEAST v.1.8.2 (Drummond *et al.*, 2012). Three independent runs with the same settings were conducted and merged subsequently with the LogCombiner utility of the same software. A GTR+G+I model with four discrete categories of rate variation, a random local clock and a Yule speciation tree prior were selected for each run. For *COI*, two different partitions were used to account for the differences in substitution rates between the first two and the third codon positions. The priors were all maintained to the default distribution and default initial values, except for relative substitution rates for which the exponential distribution was preferred because it favours convergence of independent chains. For each run, a Markov Chain of 10 000 000 generations was performed, 1 tree every 1000 sampling steps of the chain was kept (total of 10 000 trees retained) and the first 10% of the trees was discarded as the 'burn-in'. The convergence of the chains was checked with Tracer v.1.6. To test that tree topologies were not biased by heterogeneous base composition between sites, data were also analysed with Phylobayes v.4.1 (Lartillot, Lepage & Blanquart, 2009) with CAT model to account for compositional heterogeneity, using default priors for other parameters, without setting a molecular clock and using a GTR+G model with four discrete categories of rate variation. Within Phylobayes, two independent chains of 100 000 cycles were performed and merged, using a burn-in of 10% and sampling every ten states. The results were checked for good convergence of the chains, following manual guidelines.

The sequences of the four genes were concatenated with SequenceMatrix v.1.8 (Vaidya, Lohman & Meier, 2011). MP analysis of the concatenated sequences was performed with the same parameters used for the single genes, without applying any gene-specific weighting. ML analysis of the concatenated sequences was performed using RAxML v.8 (Stamatakis, 2006, 2014). Three partitions were selected as the best fitting partitioning scheme by PartitionFinder v1.1.1 (Lanfear *et al.*, 2012) and analysed independently: (1) *16S*, (2) *COI*, (3) *18S* + *28S*. A total of 100 independent inferences, using the GTRGAMMAI model and unlinked per partition estimation of branch lengths, were obtained following the RAxML manual instructions for the 'best-known likelihood tree'. BI analysis was carried out on all genes together with *BEAST (Heled & Drummond, 2010), including only species having sequences for all four genes. Priors were those used in the analyses of single genes, with the addition of a uniform prior distribution of effective population size from the root to the tips of the tree. Number and settings of runs, merging strategy and assessment of good mixing were performed in the same manner as for the single genes.

Divergence time estimation

An estimate of the divergence time between *Plutonium* and related taxa was obtained with *BEAST, without constraining the tree topology (Heled & Drummond, 2012). A calibration was applied using the times estimated by Murienne *et al.* (2010) for (1) the divergence between lithobiomorphs and scolopendromorphs (426 million years, Myr) and (2) the last common ancestor of the 'blind clade' of scolopendromorphs (296 Myr). These estimates were taken as means of normal distributions with an SD of 20 Ma. For *18S* and *28S*, an estimated rate of 0.0016 substitutions/site per Myr was set, with a permissive SD of 0.0010 substitutions/site per Myr, as previously estimated for a wide range of arthropods, including myriapods (Rota-Stabelli, Daley & Pisani, 2013).

MORPHOLOGY

We examined five adult specimens of *Plutonium*, from the four main areas from which the genus was reported: 1 ex. from the Iberian Peninsula (near Órgiva, 7 October 1977, A.L. Briganti, G. Parodi, S. Zoia leg., PD), 2 exx. from Sardinia (Pozzo n. 1 di Tres Puntas, near Galtellì, 9 February 2013, P. Marcia leg., MZ; Grotta Eliches Artas, near Oliena, 8 August 2014, J. De Waele leg., MZ), 1 ex. from the Italian Peninsula (near S. Agata sui Due Golfi, 14 May 1976, G. Osella leg., MSNVR), and 1 ex. from Sicily (near Trecastagni, 28 June 1981, P. Alicata leg., PD).

Additionally, we examined one to five adult specimens of every species of *Theatops*, with the exception of *T. chuanensis* Di *et al.*, 2010, which is known only from a single specimen but has been well described and illustrated (Di *et al.*, 2010). The specimens were: 2 exx. of *T. californiensis* Chamberlin, 1902 from California (4–7 mi north of Badger, 28 March 1941, S.D. Mulaik leg., USNM); 3 exx. of *T. erythrocephalus* (C.L. Koch, 1847) from southern Iberian Peninsula (1 ex. from near Puerto de Gáliz, 10 May 1979, A. Vigna leg., MZ; 2 exx. from near Capileira, 7–8 October 1977, A.L. Briganti, G. Parodi, S. Zoia leg., PD) and another 2 exx. of *T. erythrocephalus* from Istria (near Lovran, 25 April 1991, F. Gasparo leg., PD); 1 ex. of *T. phanus* Chamberlin, 1951 from Texas (Menard County, Powell's Cave, date and collector unknown, USNM); 2 exx. of *T. posticus* (Say, 1821) from Arizona (Roundup Camp, Madera Canyon, 11 September 1941, W. Ivie leg., USNM); and 2 exx. of *T. spinicaudus* (Wood, 1862) from Arkansas (near Lake Norfork, 2 September 1952, N. Causey leg., USNM).

All specimens were fixed in 70% ethanol. They were examined by stereo microscopy (Leica MZ12.5). Selected parts were photographed in standardized conditions, producing a stack of photographs at different focus for each subject, which were assembled in a single image using CombineZP (Hadley, 2008).

Body length, from the anterior margin of the head to the posterior tip of tergite 21, that is excluding the antennae and the ultimate pair of legs, was measured with a ruler, to the nearest 1 mm. Another 34 morphometric measures were taken under the microscope, by means of a micrometre applied to the ocular lens, at the nearest 0.1 mm. Integument features such as colour and sutures were evaluated under standardized conditions of light, with the specimens out of the preserving liquid. For the morphological terminology, we followed Bonato *et al.* (2010).

Although reliable sex assignment requires dissection and gonad examination in most scolopendromorphs, we have tentatively sexed the specimens by examining the ventral sclerites of the postpedal segments, after removing the posterior part of sternite 21. On the basis of differences observed in other scolopendromorphs, including *Cryptops* species (e.g. Pichler, 1987) and Scolopendridae (e.g. Bücherl, 1943; Klingel, 1960; Jangi, 1956, 1957), we distinguished the males for the presence of an additional medial sclerite (putatively the second genital sternite) between the first genital sternite and the less sclerotized anal laminae.

We also evaluated some characters directly on two other specimens of *P. zwierleini* (an incomplete specimen employed in the molecular analysis, see Molecular phylogenetic analysis; a dried juvenile from Sicily, Gravina di Catania, 4 October 2015, P. Galasso leg., PD) and indirectly on the photographs of another 15 specimens, either alive or fixed, taken by amateur recorders (see Geographical records).

Abbreviations for repositories are: DBCA = Dipartimento di Biologia Animale ed Ecologia, Parassitologia, Università di Cagliari; GGN = Gruppo Grotte Nuorese, Nuoro; IZCT = Istituto di Zoologia e Anatomia Comparata, Università di Catania; MNB = Museum für Naturkunde, Berlin; MSNGE = Museo civico di Storia naturale di Genova; MSNVR = Museo civico di Storia naturale di Verona; MZ = coll. M. Zapparoli; PD = coll. Bonato-Minelli, Dipartimento di Biologia, Università di Padova; USNM = National Museum of Natural History, Smithsonian Institution, Washington; ZMH = Zoologische Museum Hamburg; ZMUC = Zoologisk Museum, Statens Naturhistoriske Museum, København.

RESULTS

GEOGRAPHICAL DISTRIBUTION

Both published and new records of *P. zwierleini* are listed in Table 3 and mapped in Fig. 1. There are 24 published records, mostly documented from single specimens. In addition, we report here another 19 records, mostly supported by preserved specimens and the remaining ones by photographs. Most of the records already reported date from 1878 to 1970, while the new ones mainly date from 2008 to 2015. However, some unreported specimens were collected in the 1970s and 1980s and one in the 19th century.

All localized records cluster in four separate geographical areas in southern Europe (Fig. 1): (1) the Penibaetic system in the southern Iberian Peninsula (two localities, between Malaga and Granada); (2) Sardinia (at least 14 localities, almost all in the eastern part, in the subregions called Baronte, Barbagia, Ogliastra and Sarrabus-Gerrei, but for a few old records referred vaguely to other areas); (3) the Tyrrhenian side of the southern part of the Italian Peninsula (nine localities, most of them in the Sorrento Peninsula) and (4) Sicily (at least 11 localities, most of them in the north-eastern part).

Specimens have been found both in epigean and in hypogean habitats (caves, underground buildings) in all four areas. The elevation of the localities (reported or estimated for 18 cases) varies between a tens of metres above sea level (for all four areas) and 900 m in Sicily (Monte Gorna) and 1220 m in Sardinia (Grotta Nurra 'e Pradu, near Oliena) (Table 3).

The earliest documented records date back to 1878, or around that year, for Sardinia, the Italian Peninsula and Sicily (Cavanna, 1881, 1883; Silvestri, 1898a, b; Zapparoli, 2009), whereas they are much more recent for the Iberian Peninsula (since 1977; Table 3). The

Table 3. Documented records of *Plutonium zwierleini*, including all published and other data supported by either collected specimens or photos

Code	Locality	Date	Number of individuals	Documentation	Publication	Notes
	Penibaetic system					
1	Cueva del Tesoro, in Rincón de la Victoria	9 April 2009	1	Photo/s (by E. Cazorla Maestre)	–	–
2	Near or in Órgiva	7 October 1977	1	Specimen (A.L. Briganti, G. Parodi, S. Zoia leg., PD)	Minelli (1984), Shelley (1997)	–
	Sardinia					
3	Surroundings of Sassari	1 April 1887	1	Specimen (H. von Maltzan leg., MNB)	Edgecombe & Koch (2008)	1
4	Grotta Cane Gortoe (19 Sa/Nu), near Siniscola	7 May 2011	1	Photo/s (by C. Cilla)	–	–
5	Grotta Nurra ‘e Pradu (3083 Sa/Nu), near Oliena	9 November 2008	1	Photo/s (by L. Sanna)	Zapparoli (2009)	–
6	Grotta Eliches Artas (907 Sa/Nu), near Oliena	8 August 2014	1	Specimen (J. De Waele leg., MZ)	–	–
7	Grotta Su Guanu (103 Sa/Nu), near Oliena	September 1960	1	Specimen (A. Carta leg., GGN)	Zapparoli (2009)	–
8	Pozzo n. 1 di Tres Puntas, M. Tuttavista (1150 Sa/NU), near Galtellì	9 February 2013	1	Specimen (P. Marcia, L. Sanna leg., MZ)	–	–
9	Grotta di Istirzili (50 Sa/Nu), near Baunei	12 May 2013	1	Specimen (C. Onnis leg; MZ)	–	–
10	Near or in Asuni	30 June 1911	1	Specimen (N.L.H. Krauss leg., USNM)	Shelley (1997)	2
11	Near Aritzo	26 May 1909	1	Specimen (R. Meloni leg., MSNGE)	Zapparoli (2009)	3
12	Desulo	25 July 1883	1	Specimen	Costa (1884)	3
13	Near or in Lanusei	23 November 1899	1	Specimen (ZMH)	Würmli (1975), Shelley (1997)	4
14	Near or in Esterzili	May 2003	1	Specimen (J. Culurgioni, F. Fois leg., DBCA)	Zapparoli (2009)	–
15	Near Goni	July 2012	1	Photo/s (by C. Cabras)	–	–
16	Sarrabus	1878	1–3	Specimen/s (G.B. Traverso leg., MSNGE)	Silvestri (1898a), Zapparoli (2009)	3
17	Sarrabus	1880	1–3	Specimen/s (G.B. Traverso leg., MSNGE)	Silvestri (1898a), Zapparoli (2009)	3
	Italian Peninsula					
18	Marina di Puolo, near Massa Lubrense	15 September 1975	1	Specimen (M. Würmli leg.)	Würmli (1975)	–
19	Prasiano, near Massa Lubrense	30 August 2008	1	Photo/s (by G. Adinolfi)	–	–
20	Near or in Sant’Agata sui Due Golfi	14 May 1976	2	Specimens (G. Osella leg., MSNVR)	–	–
21	Near or in Sorrento	Before 1900	1	Specimen (J. Collin leg., ZMUC)	Meinert (1886b) [sub *Opisthemega erythrocephalum*], Shelley (1997)	5, 6
22	Fratte, near Castellamare di Stabia	4 October 2014	1	Photo/s (by A. Esposito)	–	–
23	Fratte, near Castellamare di Stabia	20 June 2015	1	Photo/s (by C. Coppola, A. Immobile, F. Fontanella)	–	–
24	Scala, near Amalfi	1927	1	Specimen (C. Wiinstedt leg; ZMUC)	Shelley (1997)	7
25	Scala, near Amalfi	1928	1	Specimen (J.E.V. Boas leg., ZMUC)	Shelley (1997)	8
26	Near or in Cava de’ Tirreni	Before 1882	1	Specimen (A. Costa leg.)	Cavanna (1883), Berlese (1884)	9
27	Near Torchiati	20 June 2015	1	Photo/s (by V. Balestrieri, S. Ferraro)	–	–
28	Maratea	10 June 2012	1	Photo/s (by I. Cammarata)	–	–

Table 3. *Continued*

Code	Locality	Date	Number of individuals	Documentation	Publication	Notes
	Sicily					
29	Near or in Palermo	16 May 1898	1	Specimen (G.A. Markens leg., ZMH)	Würmli (1975)	4, 10, 11
30	Ficuzza, south of Palermo	Before 1930	?	Specimen/s	Attems (1930)	3
31	M. Altesina – Valle dei Giunchi, near Villadoro	Before 1990	1	Specimen (R. Grasso, G. Sabella leg., IZCT)	–	12
32	Sughereta di Monte Pagano, near Caronia	Before 1990	1	Specimen (R. Grasso, G. Sabella leg., IZCT)	–	12
33	River Dittaino, near Catenanuova	5 April 1970	1	Specimen (M. Würmli leg.)	Würmli (1975)	–
34	M. Revisotto, near Troina	24 February 1964	1	Specimen (M. La Greca, S. Ruffo, G. Sichel, P. Alicata, S. Arcidiacono, V. Bianchi, S. Carfi, I. Marcellino leg., IZCT)	Matic & Dărăbanţu (1968)	3, 12
35	M. Revisotto, near Troina	March 1970	?	Specimen/s (C. Prunesco leg.)	Würmli (1975)	–
36	Probably in or near Catania	Before 2015	1	Specimen (IZCT)	–	–
37	Gravina di Catania	4 October 2015	1	Specimen (P. Galasso leg., PD)	–	–
38	Near or in Mascalucia	1 November 1973	1	Specimen (D. Caruso leg., IZCT)	–	12
39	Monte Gorna, near Trecastagni	28 June 1981	1	Specimen (P. Alicata leg., PD)	–	–
40	Near or in Taormina	1878	1	Specimen	Cavanna (1881)	3, 13
41	Near or in Taormina	1895–1896	?	Specimen/s (F. Silvestri leg.)	Silvestri (1898b)	13
42	Sicily	1881–1884	>1	Specimen/s (F. von Zwierlein or someone else leg.)	Berlese (1884)	14
43	Unknown	Before 1935	1	Specimen	Attems (1935)	15

Notes:
Records are arranged in approximate geographical order, west to east and north to south. Abbreviations for repositories: see Material and Methods.
1. The locality is indicated only as 'Sassari' on the label (J. Dunlop, Personal Communication). Such indication could actually refer not to the Sassari town but to a broader area, because at the time of the record the 'Provincia di Sassari' encompassed a large part of northern Sardinia.
2. The locality was indicated as 'Assuni' by Shelley (1997).
3. The year of the record was reported erroneously in the Italian CKmap electronic database (Zapparoli & Minelli, 2005).
4. The specimen is probably among those examined by Kraepelin (1903).
5. The specimen was misidentified and described by Meinert (1886b) as *Theatops erythrocephalus* (at the time under the genus *Opisthemega* Wood, 1862). In 1979–1980, after a suspicion expressed by A. Minelli (Personal Communication), the specimen was re-examined by H. Enghoff (Personal Communication), who recognised it as *P. zwierleini* based on the pattern of spiracles. Based on Enghoff 's identification of this specimen, Shelley (1997) listed a record of *P. zwierleini* from Sorrento merely as communicated by H. Enghoff, and Zapparoli & Minelli (2005) included this record in the Italian CKmap electronic database but, however, referring it to Meinert (1886b).
6. The date of the record is given as 1880 in the Italian CKmap electronic database (Zapparoli & Minelli, 2005), but the year should be taken as unreliable (see note 3).
7. The locality is indicated only as 'San Cataldo' on the label (H. Enghoff, Personal Communication), which is here confidently interpreted as the former Benedictine monastery in the locality Scala, in the Sorrento Peninsula. San Cataldo monastery was a property of the Danish Carl Wiinstedt from 1909 and was visited by many other Danish scientists and artists from 1924 (e.g. Lange, Pade & Waage Petersen, 2013; H. Enghoff, Personal Communication). A different interpretation was given by Shelley (1997), who referred to 'San Cataldo, ca. 6.4 km (4 mi) W Caltanissetta' (Sicily).
8. The locality is indicated only as 'Scala' on the label (H. Enghoff, Personal Communication), which is here confidently interpreted as a locality in the Sorrento Peninsula (see note 7).
9. This record from a locality in Campania (Cavanna, 1883; Berlese, 1884) was reported erroneously as from 'Calabria' by Silvestri (1898b), as clarified by Würmli (1975). The error was perpetuated by some subsequent authors: C. Attems listed *P. zwierleini* from 'Kalabrien' (Attems, 1926, 1935) or 'Calabrien' (Attems, 1930), and P. Manfredi (1956, 1957) included *P. zwierleini* in the faunal list for Calabria but not in that for Campania. This record was ignored by Kraepelin (1903) and Verhoeff (1925).
10. The locality is indicated only as 'Palermo' on the label (Würmli, 1975; Shelley, 1997).
11. The specimen was designated the neotype by Shelley (1997). It is still present in the Zoological Museum of Hamburg (K. Schütte, Personal Communication).
12. One of the two specimens showcased without label/s at the Museo di Zoologia, University of Catania (A. Marletta, Personal Communication) derives perhaps from one of these localities.
13. The provenance of the specimens was indicated only as 'Taormina' in the literature.
14. The provenance of the specimens was indicated only as 'Sicilia' by Berlese (1884), but they were possibly from near Taormina like those previously reported.
15. Attems (1935) reported to have found an incomplete specimen of *P. zwierleini* of uncertain provenance in a sample of specimens received on loan by his colleague G. Haas at the Hebrew Jerusalem University. This specimen is apparently lacking in Attems' collection in the Naturhistorisches Museum Wien (N. Akkari, Personal Communication).

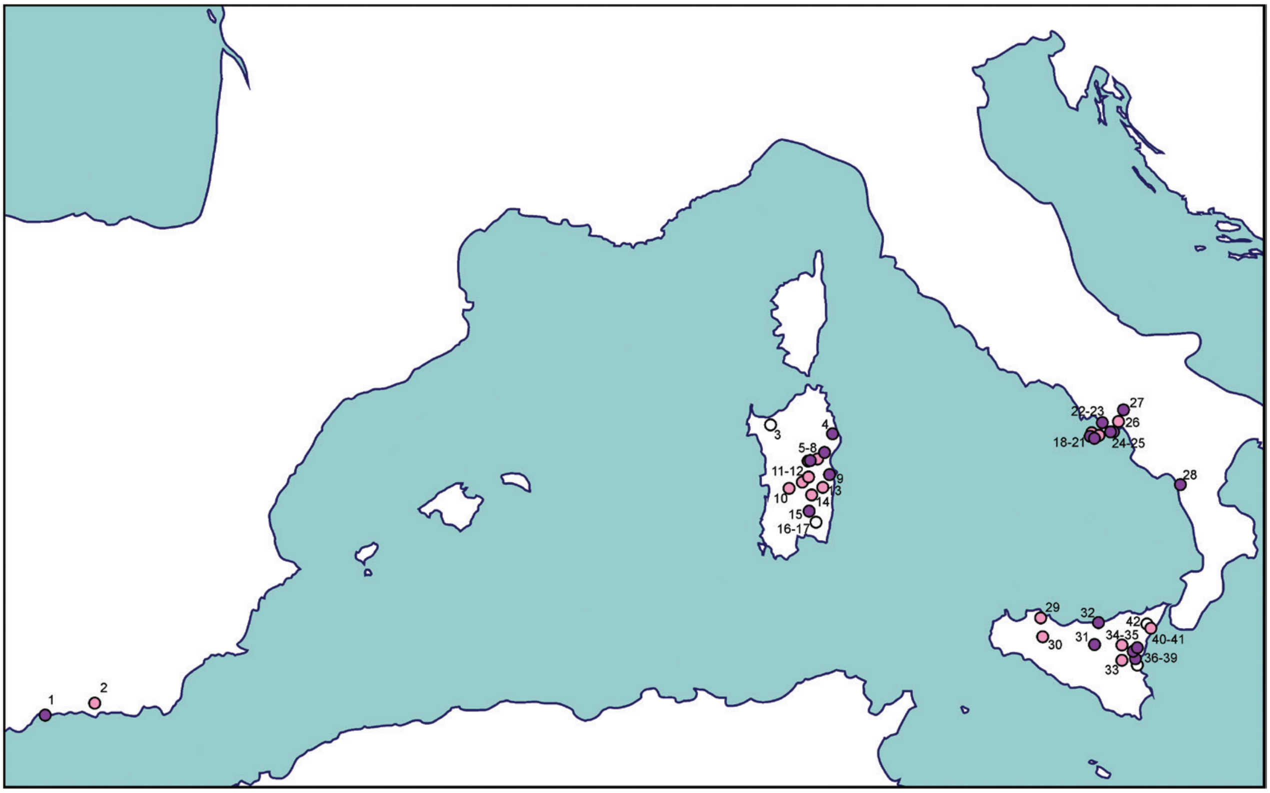

Figure 1. Geographical distribution of *Plutonium zwierleini*. Code numbers refer to records as listed in Table 3. Dark filled circles indicate new records, either documented from collected specimens or photos. Empty circles indicate old records referable only to broad areas, not to precise localities (see Material and Methods).

most recent records confirm the current presence of *P. zwierleini* in all four areas, as recent as 2009 for the Iberian Peninsula and as recent as 2015 for Sardinia, the Italian Peninsula and Sicily.

PHYLOGENETIC POSITION

Plutonium zwierleini was recovered in a well-supported monophyletic group together with the two sampled species of *Theatops* (*T. erythrocephalus* and *T. posticus*) in all phylogenetic analyses (MP, ML and BI) performed on all four genes (*16S*, *COI*, *18S* and *28S*), both when the genes were combined together (Fig. 2) and when they were considered separately (trees not shown, deposited in TreeBase: S18347).

For both species of *Theatops*, each represented by two specimens from different localities, the two specimens were confirmed to be conspecifics (Fig. 2). The within-species p-distances estimated on the *COI* sequences resulted 9.6% for *T. erythrocephalus* and 11.2% for *T. posticus*, whereas the between-species distances resulted 17.3% for the two *Theatops* species, 14.9% for *P. zwierleini* and *T. erythrocephalus* and 17.9% for *P. zwierleini* and *T. posticus*.

As to the relationships between *P. zwierleini* and the species of *Theatops*, different genes and different analytical methods support two contrasting hypotheses. *Plutonium zwierleini* was recovered to be sister of the two *Theatops* (Fig. 2A) in both MP and ML analyses of the concatenated sequences, as well as in the MP analyses of *16S* and *COI*, the ML analyses of all single genes except *COI* and the BI of *28S*. However, *P. zwierleini* was found to be more closely related to *T. erythrocephalus* than to *T. posticus* (Fig. 2B) in the BI analysis of the combined sequences, even though with low support, as well as in the MP analysis of *18S*, the ML analysis of *COI* and the BI of all single genes except *28S*. In the ML trees, the likelihood of the former hypothesis was not significantly higher than the likelihood of the latter (Shimodaira–Hasegawa test: $P > 0.05$), with the single exception of the analysis of *18S* sequences. Moreover, the results were not affected by gap removal, with the single exception of the analysis of the *16S* sequences, but also in this latter case the likelihoods of the two alternative hypotheses on the position of *P. zwierleini* resulted not significantly different (Shimodaira–Hasegawa test: $P > 0.05$).

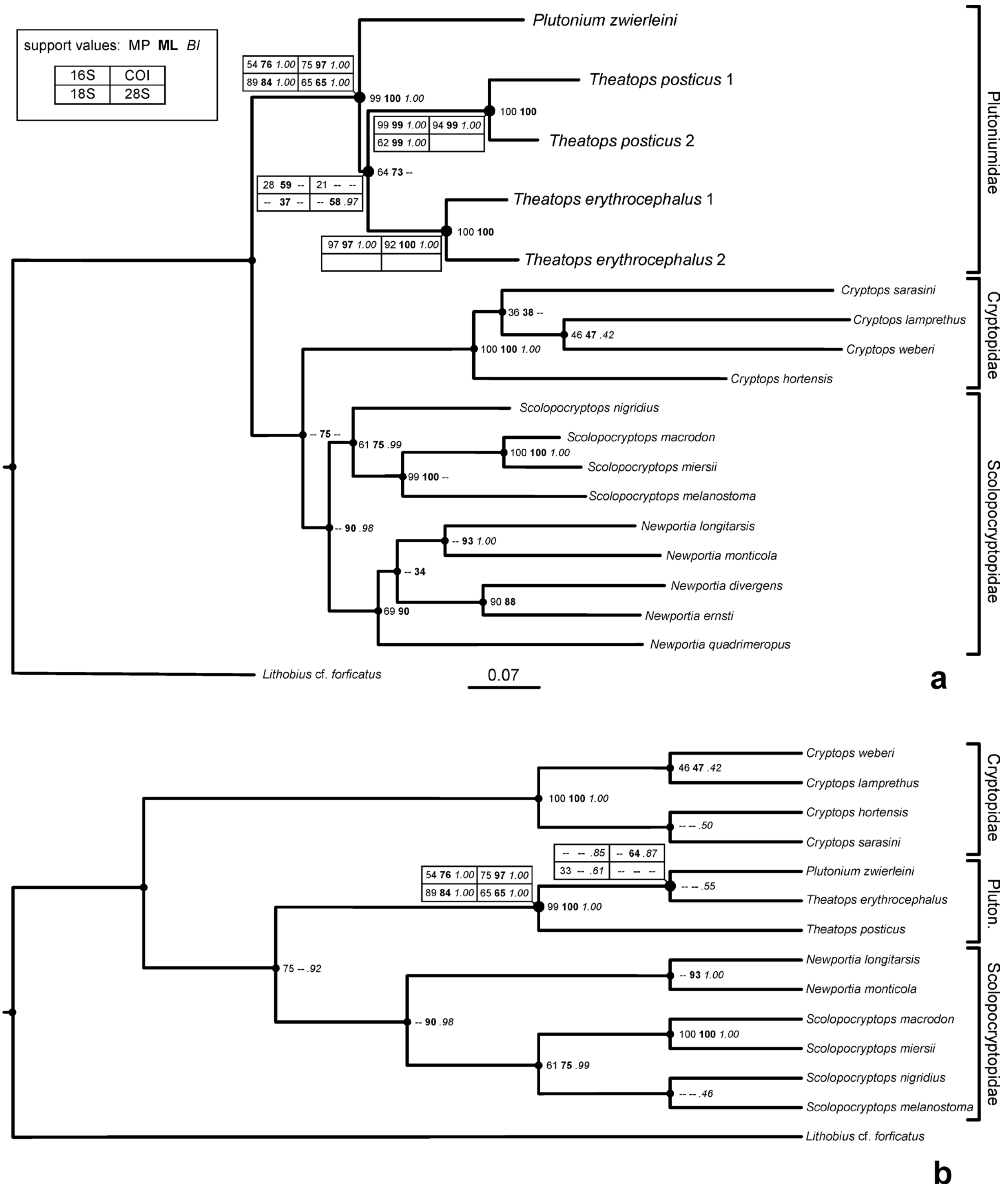

Figure 2. Phylogenetic trees obtained from the *16S*, *COI*, *18S* and *28S* sequences: (A) ML tree from the analysis of the concatenated genes; (B) tree from the Bayesian analysis of all genes combined. Numbers at nodes are bootstrap percentages obtained in MP and ML analyses, and posterior probabilities obtained from BI. For all nodes in the Plutoniumidae (marked by large circles), support values obtained from the analyses of single genes are also reported. Support values are replaced by '--' when the relevant nodes are not retrieved in the optimal tree, whereas they are fully lacking when the nodes were not evaluated because some taxa were excluded from analysis (see Table 1).

As to the relationships between the lineage *Plutonium*+*Theatops* and other lineages in the scolopendromorph ‘blind clade’, the former lineage (Plutoniumidae) was retrieved as sister to a group including all species of *Newportia* and *Scolopocryptops* (Scolopocryptopidae) in the majority of the analyses (MP of the concatenated sequences, BI of combined genes and all analyses of the single genes *16S* and *18S*). A different topology with Plutoniumidae sister to a group including all other representatives of the ‘blind clade’ (Cryptopidae and Scolopocryptopidae) is supported by the ML analysis of the concatenated sequences and the BI of the *COI*.

Assuming an age of 426 Myr for the separation between Lithobiomorpha and Scolopendromorpha, an age of 296 Myr for the last common ancestor of the ‘blind clade’ of Scolopendromorpha and an evolutionary rate of 0.0016 substitutions/site per Myr for *18S* and *28S* (see Material and Methods), we obtained an estimate of 177 Myr (95% confidence intervals: 116–236 Myr) for the separation between *Plutonium* and the two sampled species of *Theatops*, under the hypothesis that *P. zwierleini* is sister to both species of *Theatops* together (see above). Under the alternative hypothesis that *P. zwierleini* is the sister species of *Theatops erythrocephalus*, we obtained an estimate of 135 Myr ago (95% confidence interval: 63–201 Myr) for the separation between the two species.

MORPHOLOGICAL FEATURES

We found the following characters in all of the specimens of *Plutonium* and the five species of *Theatops* examined by us. Based on published sources, these characters are also shared with the single other known species of *Theatops* (*T. chuanensis*), whereas their combination does not occur in other Scolopendromorpha:

1. body colour mainly brown-orange; antennae and walking legs paler; cephalic capsule, forcipules including coxosternite, ultimate tergite and ultimate legs slightly darker (Fig. 3A);
2. head with a pair of whitish patches on the sides, at the bases of the antennae, with no vestiges of ocelli (Fig. 3B, C);
3. forcipular denticulation comprising a small tubercle at the distal end of each trochanteroprefemur and a pair of tooth plates on the coxosternite, each plate bearing up to six denticles (Fig. 3D, E);
4. 21 pairs of legs (Fig. 3A);
5. ultimate leg-bearing segment bearing a subrectangular tergite distinctly longer than the penultimate tergite (Fig. 3A), an elongate subtrapezoidal sternite with slightly bilobate posterior margin (Fig. 4A, B), and telopodites conspicuously swollen and tapering into piercing tips (Fig. 4C, D).

We found the following traits in all the examined specimens of *Plutonium*, from all the four geographic areas (southern Iberian Peninsula, Sardinia, Sicily, southern Italian Peninsula), but missing in all species of *Theatops* (Table 4):

1. body length exceeding 80 mm (with the only exception of 3 juveniles out of 14 measured specimens) and up to 120 mm (with even higher figures reported in the literature; see Discussion); the greatest lengths of the species of *Theatops* are in the range of 50–80 mm;
2. cephalic plate with elongate longitudinal sutures extending to the anterior half of the head (Fig. 5A, B); such sutures are either wanting or distinctly shorter in the species of *Theatops*;
3. 19 pairs of spiracles along the trunk, one pair on every leg-bearing segment from 2 to 20 (Fig. 5E–H); in contrast, only 9–10 pairs of spiracles are present in all *Theatops* species, on approximately alternate segments;
4. clusters and bands of dense setae at the bases of most legs, in particular on procoxa, metacoxa and ventral sides of the prefemora on all legs from pair 1 to 17–18 and on the ventral side of the femora on all legs from pair 1–2 to 12–17 (Figs 5C, D, 6D); these setae, which are about 180 µm long, are distinctly longer and denser than the ordinary setae, which are up to 50 µm long and are scattered on the remaining surface of the legs and the trunk; only ordinary setae are present in the same position in all species of *Theatops*;
5. posterior part of trunk enlarged, that is, the leg-bearing segments gradually increasing in size along the trunk, anteriorly to posteriorly, more conspicuously in *Plutonium* than in *Theatops* (Fig. 3A);
6. claws of the legs of the ultimate pair very elongated and with a ventral blade, articulated on relatively stout articles (Fig. 6F), that is, the pretarsi proportionally longer in *Plutonium* than in *Theatops* when compared to the other articles, the tarsi instead shorter, and the pretarsus expanding ventrally in a sclerotized ridge instead of being uniformly tapering and approximately circular to elliptical in crosssection as in the species of *Theatops*.

In addition, the appendages (especially the antennae, but also all the legs including the ultimate pair) are relatively elongated in *Plutonium* (Figs 5C–H, 6A), that is proportionally longer with respect to the body, more slender and more strongly tapering than in most species of *Theatops*, with the notable exception of *T. phanus* (Table 4). The forcipular coxosternite projects less and the tooth plates are stouter in *Plutonium* than in most species of *Theatops* (Fig. 3D, Table 4). Also, no sclerotized dark spines were found on the

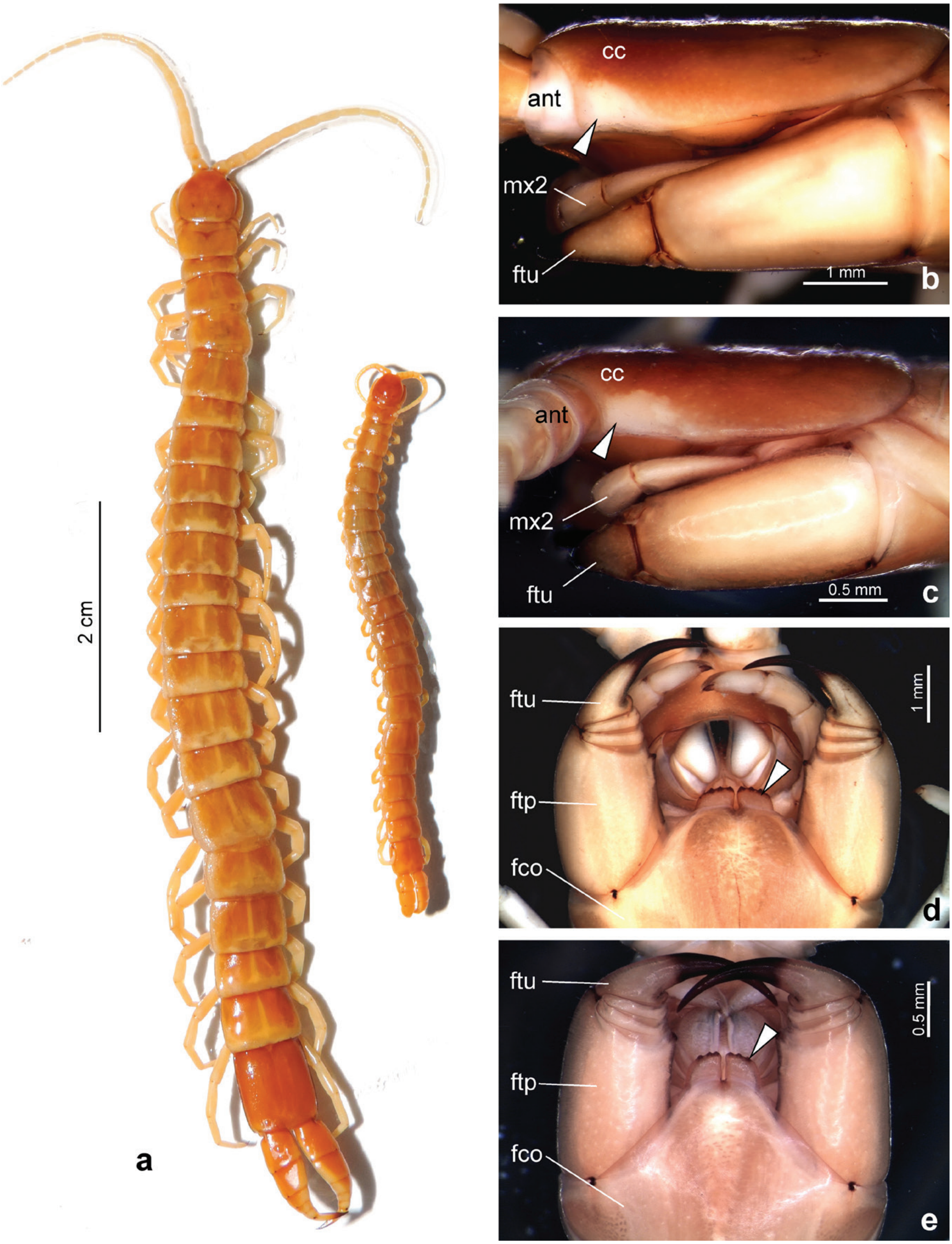

Figure 3. *Plutonium zwierleini* (A left, B, D) and *Theatops erythrocephalus* (A right, C, E): (A) entire body, dorsal view; (B, C) head and forcipular segment, left lateral view; (D, E) forcipular segment, ventral view. Specimens: *P. zwierleini* from Sicily, near Trecastagni; *T. erythrocephalus* from Istria, near Lovran (full data in Material and Methods). Abbreviations: ant, antenna; cc, cephalic capsule; fco, forcipular coxosternite; ftp, forcipular trochanteroprefemur; ftu, forcipular tarsungulum; mx2, telopodites of second maxillae. Arrowheads: (B, C) ocellar area; (D, E) tooth plate.

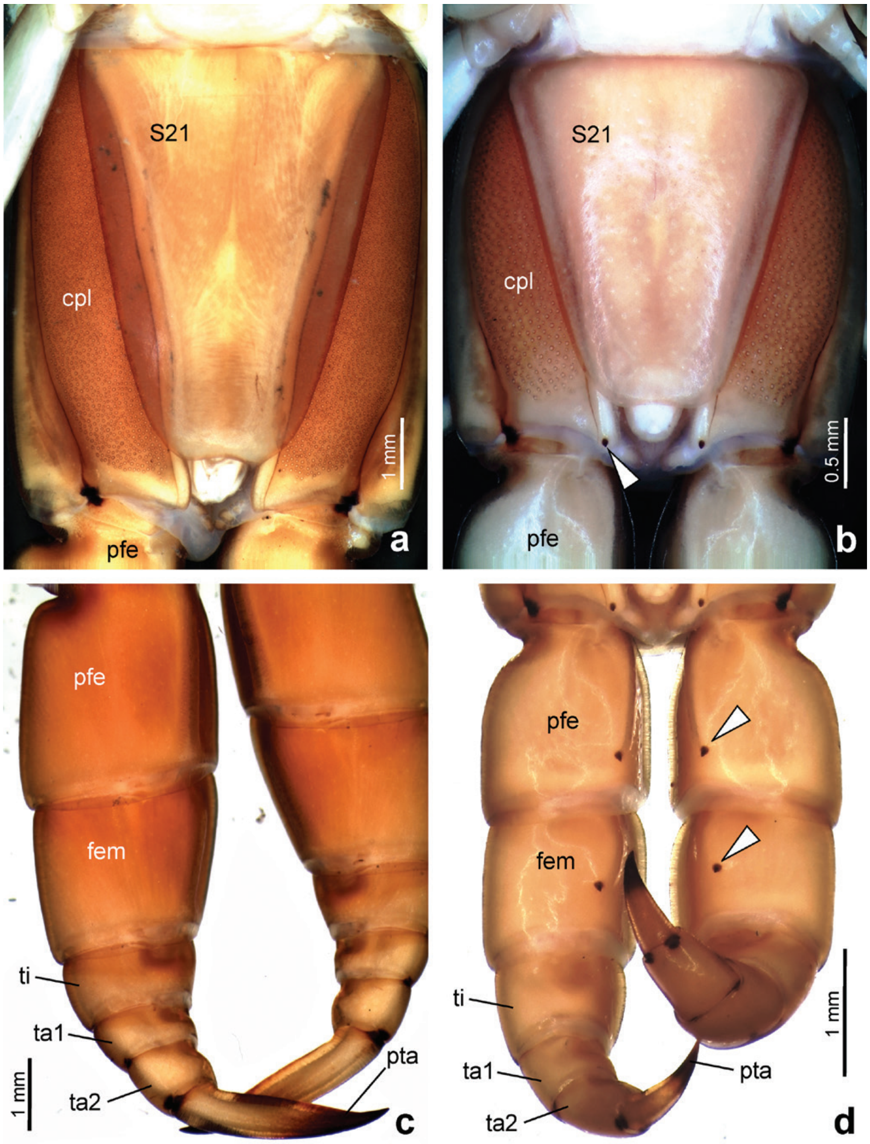

Figure 4. *Plutonium zwierleini* (A, C) and *Theatops erythrocephalus* (B, D): (A, B) leg-bearing segment 21, ventral view; (C, D) legs 21, ventral view. Specimens as in Fig. 3. Abbreviations: cpl, coxopleuron; fem, femur; pfe, prefemur; pta, pretarsus; S21, sternite 21; ta1, tarsus 1; ta2, tarsus 2; ti, tibia. Arrowheads: spines.

ultimate leg-bearing segment of *Plutonium*, neither on the posterior tips of the coxopleura (Fig. 4A) nor on the basal articles of the legs (Fig. 4C), whereas spines of this kind are often present in the species of *Theatops*, although in variable number and position both within and between species (Fig. 4B, D).

DISCUSSION

GEOGRAPHICAL DISTRIBUTION

Previous knowledge of the geographical distribution of *Plutonium* was based on only 24 records, most dating back several decades. Moreover, it was flawed by the imprecise localization of some records, by doubts about the geographical provenance of some specimens and by hitherto unnoticed errors in interpreting or citing geographical names (see notes in Table 3).

Our survey has nearly doubled the total number of records (Table 3), proving that populations of *P. zwierleini* are still extant not only in Sardinia (Zapparoli, 2009) but also in the Italian Peninsula and in Sicily, where the last published records dated from 1975 and 1970, respectively (Würmli, 1975). In addition, residual doubts on the occurrence of *Plutonium* in the Iberian Peninsula have been removed by the

Table 4. Differences between *Plutonium* and *Theatops* species

	P. zwierleini	*T. californiensis*	*T. chuanensis*	*T. erythrocephalus*	*T. phanus*	*T. posticus*	*T. spinicaudus*
Sources	Original	Original; Chamberlin, 1902; Shelley, 1997, 2002	Di *et al.*, 2010	Original; Latzel, 1880; Verhoeff, 1896, 1901; Machado, 1952; Matic, 1960	Original; Chamberlin, 1951; Shelley, 1997, 2002	Original; Meinert, 1886a (as *Opisthemega crassipes*); Kraepelin, 1903; Shelley, 1987, 1990, 1997, 2002	Original; Wood, 1865; Meinert, 1886a; Kraepelin, 1903; Shelley, 1987, 1997, 2002
Specimens reported	10–50	>100	1	>100	10–50	>100	>100
Specimens examined by us	5	2	0	5	1	2	2
Distribution	Southern Europe	Western North America	Central Asia	Southern Europe	Western North America	Western and eastern North America	Eastern North America
Habitat	Epigean and hypogean	Epigean	Epigean	Epigean and hypogean	Epigean and hypogean	Epigean	Epigean
Body: maximum length	120 mm[a]	78 mm	70 mm[b]	61 mm	64 mm	50 mm	50 mm
Head: posterior paramedian sutures	Present, reaching head mid-point at least	Usually present, not reaching head mid-point	Present, not reaching head mid-point	Usually absent, or very short	Usually absent, or very short	Usually absent, or very short	Usually absent, or very short
Antenna length/ head breadth	3–6	3–4	3–4	2–3	5–6	2–3	3–4
Antenna: article XVII/article II width	0.1–0.3	0.3–0.4	~0.3	0.3–0.4	0.1–0.2	0.3–0.4	0.2–0.3
Forcipular tooth plate: length/width	0.4–0.7	0.6–0.7	~0.9	0.8–1.0	0.7–0.8	0.6–0.7	0.7–0.9
Forcipular tooth plate: number of teeth	3–4	3–4	1–2	3–4	0–2	2–5	2–4
Leg-bearing segments with spiracles	Segments 2–20	Segments 3,5,8,10,12, 14,16,18,20	Segments 3,5,7,8,10,12, 14,16,18,20	Segments 3,5,8,10,12, 14,16,18,20	Segments 3,5,8,10,12, 14,16,18,20	Segments 3,5,8,10,12, 14,16,18,20	Segments 3,5,8,10,12, 14,16,18,20
Leg 4: tibia length/ width	1.9–3.7	~2.9	~2.9	1.9–2.1	3.5–3.7	2.3–3.1	2.3–2.8
Legs and pleurites: dense, long setae	Present	Absent	Absent?	Absent	Absent	Absent	Absent
Tergite 20/tergite 1 length	>1.4	<1.4	<1.4	<1.4	<1.4	<1.4	<1.4
Tergite 21: mid-longitudinal suture	Present, complete	Present, almost complete	Present, almost complete	Present, almost complete	Present, complete	Present, complete	Absent, or very short

Table 4. *Continued*

	P. zwierleini	*T. californiensis*	*T. chuanensis*	*T. erythrocephalus*	*T. phanus*	*T. posticus*	*T. spinicaudus*
Leg 21: coxopleuron: distal spine	Absent	Present	Present	Present	Absent	Absent	Absent
Leg 21: prefemur: dorsal spine	Absent	Absent	Absent	Absent	Present	Absent	Present
Leg 21: prefemur and femur: ventral spines	Absent	Present	Absent	Present	Absent or present[c]	Absent or present[c]	Absent or present[c]
Leg 21: tarsus 2/ prefemur length	0.2–0.3	~0.6	~0.4	0.3–0.5	0.3–0.5	0.3–0.4	0.3–0.4
Leg 21: pretarsus/ prefemur length	>0.8	<0.7	<0.7	<0.7	<0.7	<0.7	<0.7

Notes:
[a] Higher figures reported in the literature are questionable (see Discussion).
[b] The length reported by Di *et al.* (2010), that is 76.6 mm, included the ultimate legs and has been corrected based on the photos published in the same paper.
[c] The intraspecific variation is described in detail by Shelley (1990, 1997).

direct examination of the single specimen previously reported (Shelley, 1997) and by another documented record in a nearby locality. Conversely, uncertainty remains on the identity and provenance of a specimen found in a collection on loan from the University of Jerusalem (Attems, 1935; see note 15 of Table 3).

As to the ecology and habits of *Plutonium*, most reports lack detail and past speculation was mainly based on the functional interpretation of the species' anatomical features (e.g. Manton, 1965; Schileyko, 1992, 1996). Old and recent records refer to both epigean (soil or superficial rocky debris; woods, maquis, pastures and also urban settlements and cultivated land) and hypogean sites (natural caves, at least in the Iberian Peninsula and in Sardinia, but also buildings, especially basements and ground floors). It is worth noting that some species of *Theatops* may also colonize caves (Table 4). These include the European *T. erythrocephalus* (e.g. Ribaut, 1915; Matic, 1960; Würmli, 1975; Serra, 1983) and the American *T. phanus* (Shelley, 1997).

Phylogeny and evolution

The phylogenetic position of *Plutonium* within the scolopendromorph centipedes has been a contentious issue until recent times. Some unusual traits (especially the continuous series of spiracles along the body and the forcipule-like ultimate legs), along with other features that have been arbitrarily emphasized (the number of leg pairs and the fine morphology of the spiracles), induced different authors to propose contrasting evolutionary hypotheses and taxonomic schemes. When first discovered, *Plutonium* was originally presented as most probably closer to some scolopendrids currently distinguished as Otostigmini (Cavanna, 1881, also in Berlese, 1884). Soon after, by overemphasizing the unique arrangement of the spiracles, *Plutonium* was separated from all other scolopendromorphs as a distinct lineage labelled 'Skolopendriden holopneusticae' by Haase (1884) and 'Plutoninae' by Bollman (1893a, b). However, a closer relation with *Theatops* was first endorsed by P. Bertkau (Meinert, 1886a) and Haase (1887), and this view has subsequently been reflected in most of the otherwise variable taxonomic systems elaborated during the 20th century. Different authors grouped *Plutonium* with *Theatops* and both genera with the Cryptopidae rather than the Scolopendridae (Pocock, 1896; Kraepelin, 1903; Verhoeff, 1907; Attems, 1926, 1930; Prunesco, 1970a, 1997; Bücherl, 1971; Shelley, 1997). Kraepelin (1903) explicitly suggested that the anatomical features of *Plutonium* were somehow derived from those of *Theatops*. However, a radically different view was first elaborated by Verhoeff (1906, 1907), who considered *Plutonium* as resembling

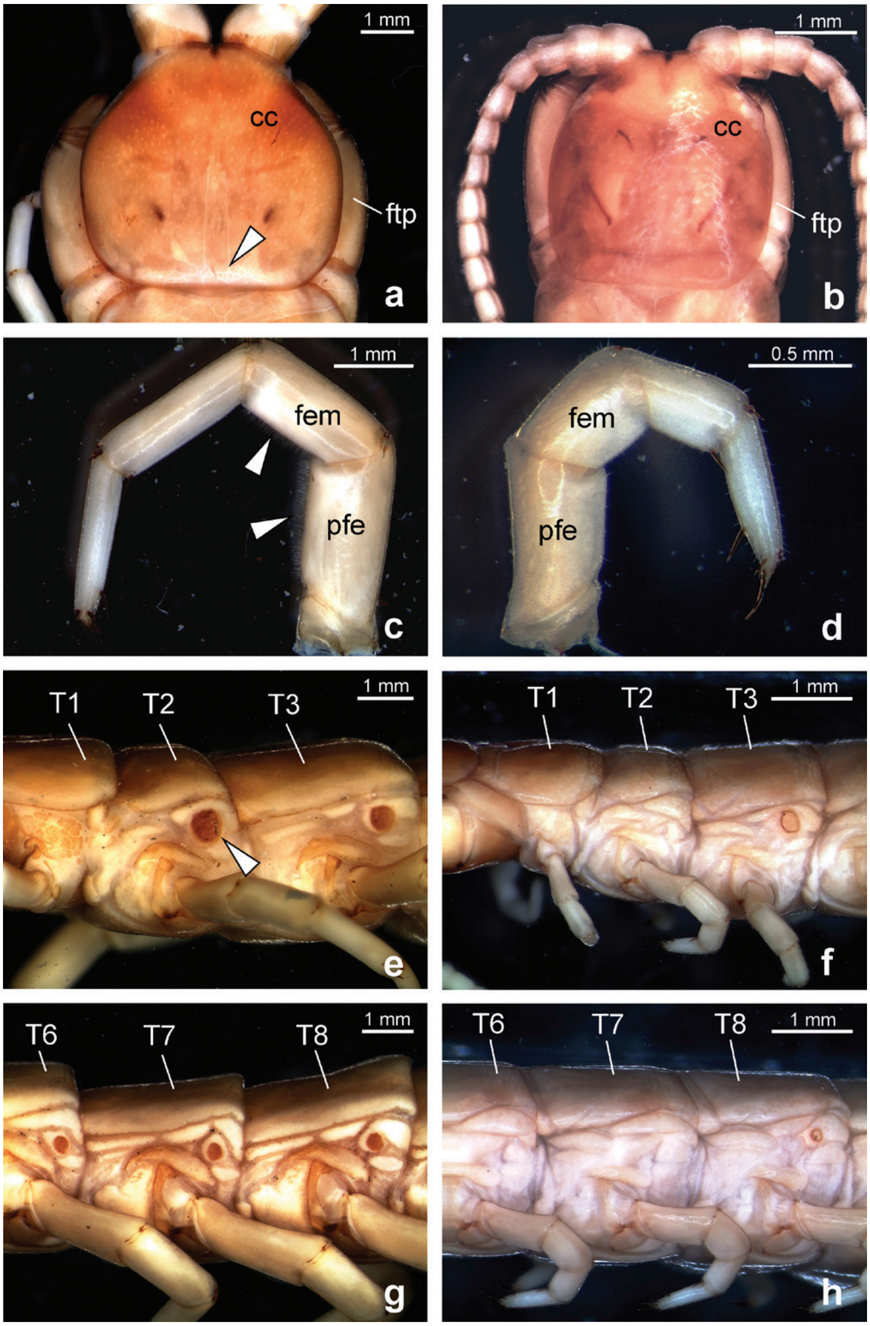

Figure 5. *Plutonium zwierleini* (A, C, E, G) and *Theatops erythrocephalus* (B, D, F, H): (A, B) head, dorsal view; (C, D) leg 4, anterior view; (E, F) anterior leg-bearing segments, left lateral view; (G, H) leg-bearing segments from mid-trunk, left lateral view. Specimens as in Fig. 3. Abbreviations: cc, cephalic capsule; fem, femur; ftp, forcipular trochanteroprefemur; pfe, prefemur; TX, tergite X. Arrowheads: a, suture; c, dense, long setae; e, spiracle 2.

a hypothetical ancestor (called '*Proplutonium*') of both *Theatops* and the Scolopendridae, but not of the Cryptopidae. Such view was eventually abandoned by the same author (e.g. Verhoeff, 1925) to be later resurrected and further developed by Schileyko (1992, 1996; Schileyko & Pavlinov, 1997), who regarded *Plutonium* as deeply separated from all other scolopendromorphs because of its supposedly ancestral characters.

In the two last decades, a strict relationship between *Plutonium* and *Theatops* has been corroborated by morphological examinations (Shelley, 1997, 2002) and cladistic analyses, also on the basis of newly acquired

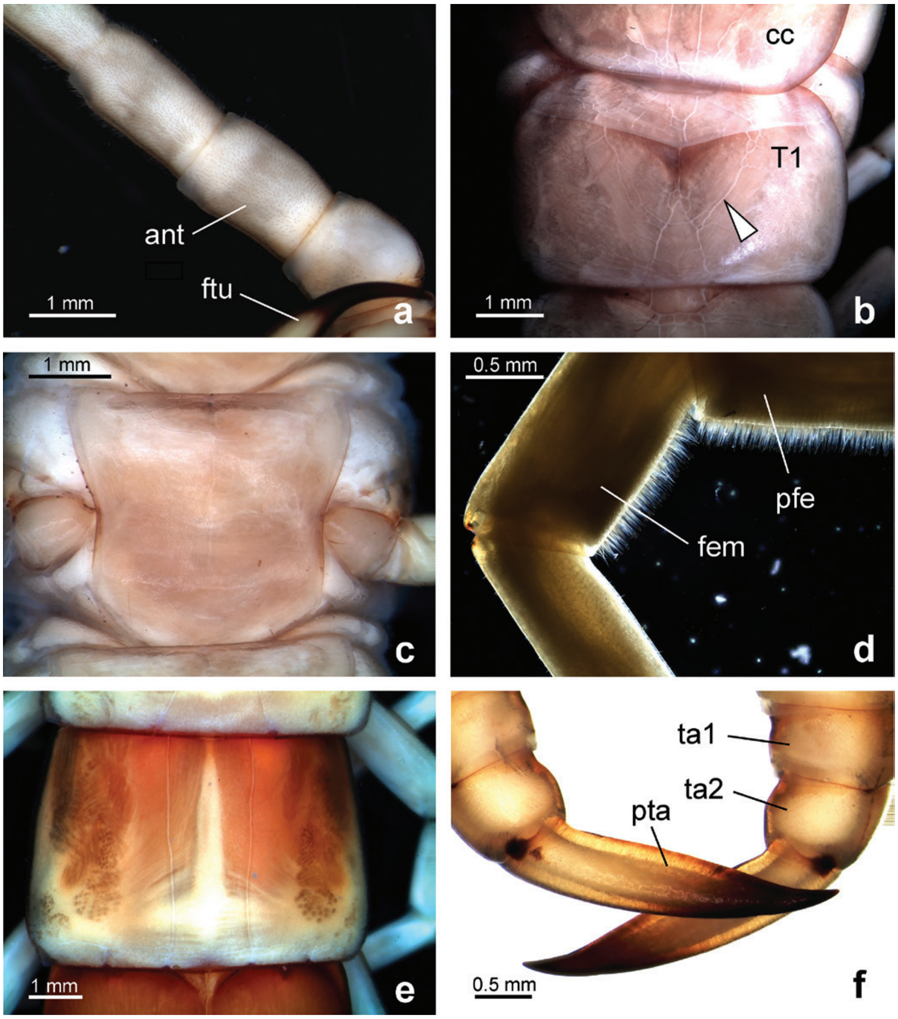

Figure 6. *Plutonium zwierleini*: (A) basal part of right antenna, ventral view; (B) tergite 1, dorsal view; (C) sternite 4, ventral view; (D) part of right leg 4, anterior view; (E) tergite 20, dorsal view; (F) pretarsi of legs 21, dorsal view. Specimens as in Fig. 3. Abbreviations: ant, antenna; cc, cephalic capsule; fem, femur; ftu, forcipular tarsungulum; pfe, prefemur; pta, pretarsus; T1, tergite 1; ta1, tarsus 1; ta2, tarsus 2. Arrowhead: suture.

anatomical evidence (Edgecombe &Koch, 2008, 2009; Koch *et al.*, 2009; Koch, Edgecombe & Shelley, 2010; Vahtera et al., 2012b, 2013). This view is confirmed here by the first phylogenetic analysis based on molecular data.

Based on this phylogenetic hypothesis, the following characters, shared by *Plutonium* and all known species of *Theatops*, but not by their closest relatives, were most probably already present in their last common ancestor: light patches on the sides of the head (Fig. 3B, C), sclerotized tooth plates on the forcipular coxosternite (Fig. 3D, E) and the ultimate leg-bearing segment with a broad tergite, an elongate sternite and forcipule-like swollen legs (Fig. 3A). However, tooth plates and somehow similar ultimate legs are not exclusive of *Plutonium* and *Theatops*, having evolved independently also within the distantly related Scolopendridae. In addition to these external characters, other anatomical features have been proposed as synapomorphies of *Plutonium* and *Theatops*, even though only selected species of *Theatops* have been examined: calyx of the forcipular poison gland deepening into the coxosternite (Edgecombe & Bonato, 2011); spiracles lined with trichomes that are irregularly flattened and reticulate, rather than elongate (Vahtera *et al.*, 2012b); and gizzard provided with sieve projections covered with multifurcating scales (Koch *et al.*, 2009).

As to the relationships between *Plutonium* and *Theatops*, our molecular data do not decisively favour any of the two alternative hypotheses: (1) *Plutonium* and *Theatops* represents two separate lineages, which is consistent with a previous hypothesis elaborated on morphological similarities (Shelley, 1997) and in agreement with current taxonomy, (2) *Plutonium* is a derived lineage within *Theatops*, which is supported also by previous cladistic analyses on morpho-anatomical data, although with *P. zwierleini* closer to *T. posticus* rather than to *T. erythrocephalus* (Edgecombe & Koch, 2008, 2009; Koch *et al.*, 2009, 2010; Vahtera *et al.*, 2012b). Further analyses including other species of *Theatops* will be necessary to test the two alternative scenarios, with possible consequences on the current taxonomy.

MORPHOLOGY

By examining multiple specimens of *Plutonium*, we clarified some characters that had previously been reported either inconsistently or ambiguously. The body colour, for instance, is actually quite uniformly brown-orange, both in living specimens (Fig. 7) and after preservation in ethanol, even after many decades (Fig. 3A). However, the first specimens of *Plutonium* had been described as yellow-olive when fixed in liquid (Cavanna, 1881; Berlese, 1884) and the first specimen illustrated in a colour plate appeared even more brown-olive (Berlese, 1884), and as such was described by Attems (1926). In contrast, newly examined specimens were reported by Kraepelin (1903) as either yellow-red or brown-olive and the colour was more accurately described as yellow-orange only in Berlese (1925), as confirmed in recent years by the first photograph of a living individual (Zapparoli, 2009).

As to the maximum body size reached by *Plutonium*, our knowledge still rests on poor data. Out of 14 specimens measured, the longest was found to be 120 mm long from the anterior margin of the head to the tip of the ultimate tergite. Higher figures can be found in the literature, including a maximum of 140 mm first reported by Kraepelin (1903) and later by others (Attems, 1930; Bücherl, 1971) and a limit of 150 mm given in some recent compilations (Di *et al.*, 2010; Edgecombe & Bonato, 2011). However, we cannot rule out the possibility that Kraepelin's measure was actually taken on the full body size of a specimen, including the ultimate legs, while Di *et al.*'s figure may derive from a rough approximation of previously available data (G. Edgecombe, Personal Communication). In any case, to the best of our knowledge, *P. zwierleini* has a larger body than any species of *Theatops*, which can be estimated to reach a maximum body length between 5 and 8 cm (Table 4). Among the European scolopendromorph centipedes, only *Scolopendra cingulata* can be as large as *P. zwierleini*; however, specimens exceeding 12 cm in length are reputedly rare and specimens up to 16 cm have only been reported from south-eastern Europe (Kraepelin, 1903; Simaiakis, Giokas & Korsós, 2011).

Some of the unique features of *P. zwierleini*, like the continuous series of spiracles along the body and the blade-like claws of the ultimate legs, have long been known. However, other distinctive features, like the conspicuous sutures on the head and the elongation of the appendages in comparison to most species of *Theatops*, have not previously been noticed.

Also the clusters and stripes of dense long setae present on the legs and the adjacent pleurites (Fig. 6D) seem to have been completely overlooked by all previous authors, as they have been neither mentioned

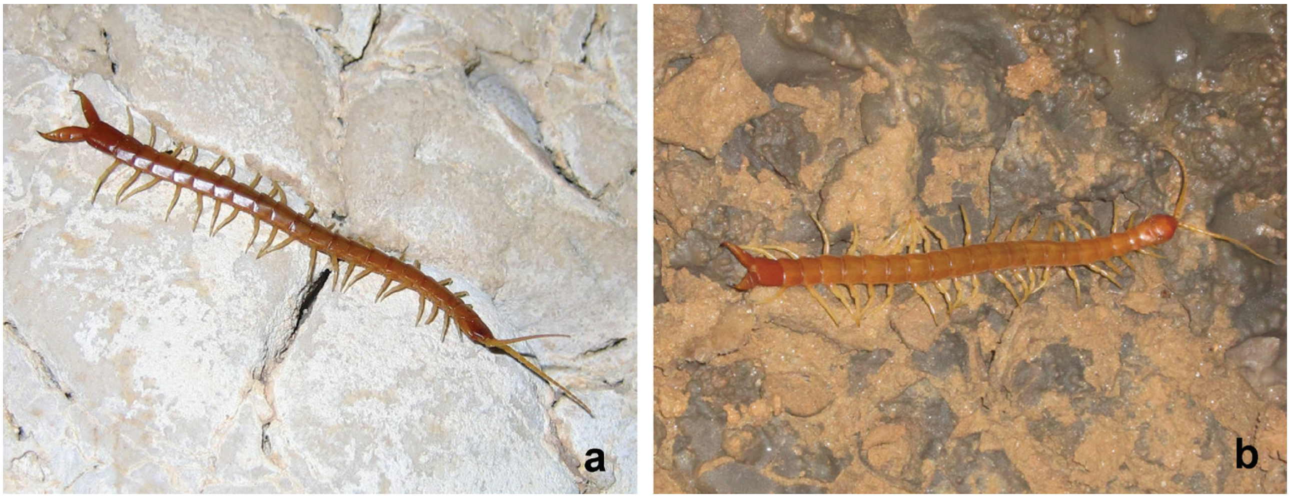

Figure 7. *Plutonium zwierleini*. Photos of living specimens taken in the field: (A) Iberian Peninsula, Cueva del Tesoro, near Rincón de la Victoria, 9 April 2009, photo by E. Cazorla Maestre; (B) Sardinia, Cane Gortoe cave, near Siniscola, 7 May 2011, photo by C. Cilla.

nor illustrated before. We detected these dense long setae in all five specimens of *Plutonium* examined and also in another specimen through high-quality photographs, but in none of the examined specimens of *Theatops*. Additionally, no evidence comes from the literature that similar setae have ever been found in any species of *Theatops*. The arrangement of these setae along the trunk is much the same in all specimens of *P. zwierleini*, independent of body size or the presumed sex. Similar setae, or other structures in the same position, are unknown in other scolopendromorphs and their function is hard to conjecture.

No evidence of sexual dimorphism has emerged from comparing two presumed males with three presumed females. Indeed, when reporting on a sample of specimens of *P. zwierleini* collected in Sicily, Cavanna (in Berlese, 1884) speculated on some differences between sexes to account for the fact that the ultimate legs appeared more swollen, and the corresponding sternite longer, in some specimens than in others. It is worth noting, however, that sexual dimorphism in external characters is very rare in scolopendromorphs and very few compelling cases have been reported so far (Bücherl, 1943; Lewis, 1968; Jangi & Dass, 1975; Simaiakis *et al.*, 2011).

One of the most remarkable features of *Plutonium* is the fact that the ultimate legs closely resemble the anterior poisonous forcipules (Figs 4C vs. 3D). The similarities between these appendages at the opposite ends of the body include not only their overall shape, size and integumental robustness, but also features affecting the way they can be moved. Comparable adduction/abduction movements can be inferred from the similar position of their insertion with respect to the main body axis, the proportions between the articles and the orientation of the hinges. The general shape of the ultimate legs is common to *Theatops* and *Plutonium*, but in the latter the tarsal articles are relatively shorter and the claw longer and distinctly blade-shaped (Fig. 4C, D). Among other scolopendromorphs, the ultimate legs are conspicuously swollen only in a few small lineages of Scolopendridae, which include *Asanada* and *Asanadopsis* (e.g. Jangi & Dass, 1984), *Sterropristes* (Muadsub *et al.*, 2012) and a subgroup of species of *Cormocephalus* (e.g. Shelley, 1997). However, really similar, so-called forcipulate legs are present only in *Scolopendropsis* (Chagas-Junior, Edgecombe & Minelli, 2008). Lacking behavioural observations, we can only speculate that the ultimate legs of *Plutonium* are employed in defence against predators, as commonly observed in many other scolopendromorph centipedes. In the few specimens of *Plutonium* photographed alive, the ultimate legs were often raised and splayed (Fig. 7), resembling the warning posture displayed by some scolopendrids when disturbed (Kronmüller & Lewis, 2015). It has also been speculated that scolopendromorphs can use the ultimate legs to catch and hold prey (e.g. Bücherl, 1971), a hypothesis also explicitly advanced for the forcipule-like legs of *Theatops* (e.g. Cloudsley-Thompson, 1958; Shelley, 1997) and *Plutonium* (Manton, 1965), but there are no direct observations in these or other centipedes to support this (Lewis, 2010).

We found no evidence of variation between *Plutonium* specimens from different areas and habitats in major characters like body size, colour, overall shape, length and density of setae, pattern of sutures on the tergites and spurs on the walking legs. However, some variation seems to occur in the elongation of the appendages, either related to habitat differences or to geographic differentiation: out of the ten specimens for which it was possible to estimate the relative length of the antennae, five specimens from Sardinian caves have more elongate antennae (about five times the length of the head) than five specimens found in epigean habitats in the Italian Peninsula (three to four times the length of the head). It is worth noting that while *T. erythrocephalus* specimens from an Iberian cave do not show evident morphological differences from specimens of the same species collected in epigean sites (Serra, 1983), cave-dwelling specimens of *T. phanus* markedly differ in colour, body size and elongation of antennae and legs from the specimens living outside the caves (Shelley, 1997).

Further investigations of morphology of a larger sample of specimens and of genetic variation between populations of *Plutonium* are required to assess the differences between geographical areas and different habitats.

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