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A new stick-insect of hybrid origin: Bacillus lynceorum n. sp. (Cheleutoptera: Bacillidae)

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Zoologia. — *A new stick-insect of hybrid origin*: Bacillus lynceorum *n. sp.* (*Cheleutoptera: Bacillidae*) (*). Nota di LUCIANO BULLINI (**), GIUSEPPE NASCETTI (**) e ANNA PAOLA BIANCHI BULLINI (**), presentata (***) dal Socio G. MONTALENTI.

RIASSUNTO. — Viene descritto un fasmide nuovo per la scienza: *Bacillus lynceorum* n. sp., rinvenuto recentemente nella Sicilia meridionale. Sono indicati i caratteri morfologici che permettono il riconoscimento di questa specie e viene descritto il suo corredo cromosomico e la sua struttura genica sulla base dello studio elettroforetico di venti loci enzimatici. La riproduzione di *B. lynceorum* avviene per partenogenesi telitoca obbligatoria e non sono stati fino ad ora osservati maschi. I dati cariologici ed elettroforetici indicano che *B. lynceorum* è derivato dall'ibridazione di *B. grandii*, specie bisessuata, e e di *B. whitei*, specie partenogenetica anch'essa di origine ibrida. L'ibridazione non è stata accompagnata dal raddoppiamento del numero cromosomico. Nel cariotipo di *B. lynceorum* coesistono il corredo cromosomico aploide di *B. grandii* e quello diploide di *B. whitei*. La condizione allotriploide risulta confermata a livello elettroforetico.

INTRODUCTION

Animal speciation by hybridization was considered, until recently, as a rare event (White [8]). The combined use of electrophoretic and karyological techniques has allowed us to discover in recent years a number of species of hybrid origin in different animal groups, such as snails, beetles, and stick-insects (Nascetti and Bullini [4]; Bullini [1], [2]; Magnano *et al.* [3]). In the last group, speciation by hybridization seems to have occurred rather frequently: three out of the five stick-insects recorded up to now in Italy: *Bacillus atticus* Brunner, *B. whitei* Nascetti and Bullini, and *Clonopsis gallica* (Charp.), have a hybrid origin (Bullini [1], [2]; Nascetti and Bullini [5], [6]; Nascetti *et al.* [7]).

In the present paper a new hybrid species of stick-insect from Sicily is described at the morphological, karyological and electrophoretic level; its ancestors are identified, and the evolutionary events involved in its speciation are discussed.

Bacillus lynceorum n. sp.

Material examined – Holotype: ♀, surroundings of Santo Pietro, m 280 (Catania), September 25, 1982, L. Bullini and G. Nascetti leg. Preserved at the Civic Natural History Museum " Giacomo Doria " in Genoa. Paratypes:

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(***) Nella seduta del 23 giugno 1983.

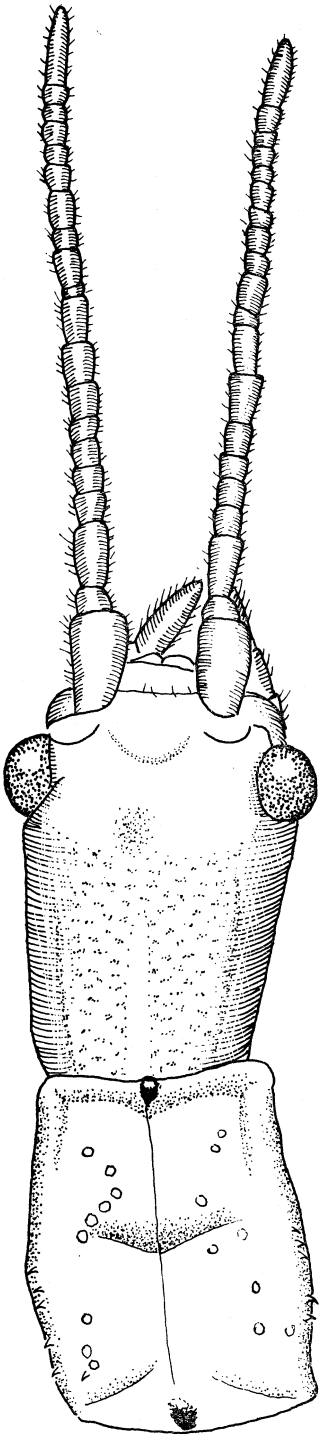

Fig. 1. – *Bacillus lynceorum* from Santo Pietro (Sicily): head and pronotum: dorsal view.

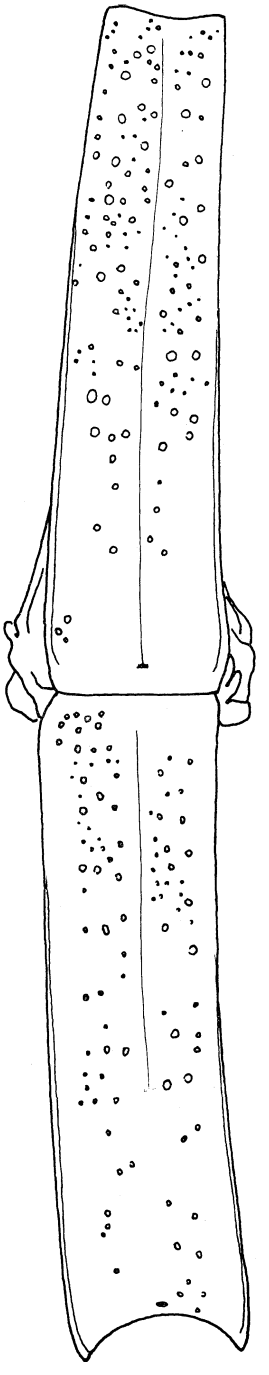

Fig. 2. – *Bacillus lynceorum* from Santo Pietro (Sicily): meso– and metanotum: dorsal view.

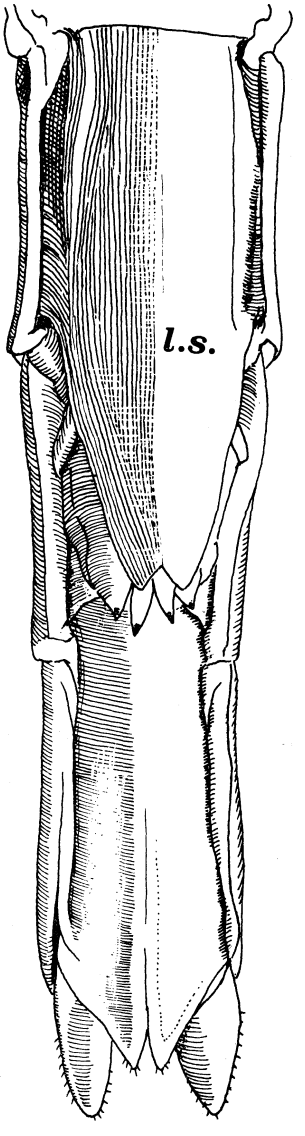

Fig. 3. – *Bacillus lynceorum* from Santo Pietro (Sicily): end of abdomen (ventral view), showing the lamina subgenitalis (*l.s.*).

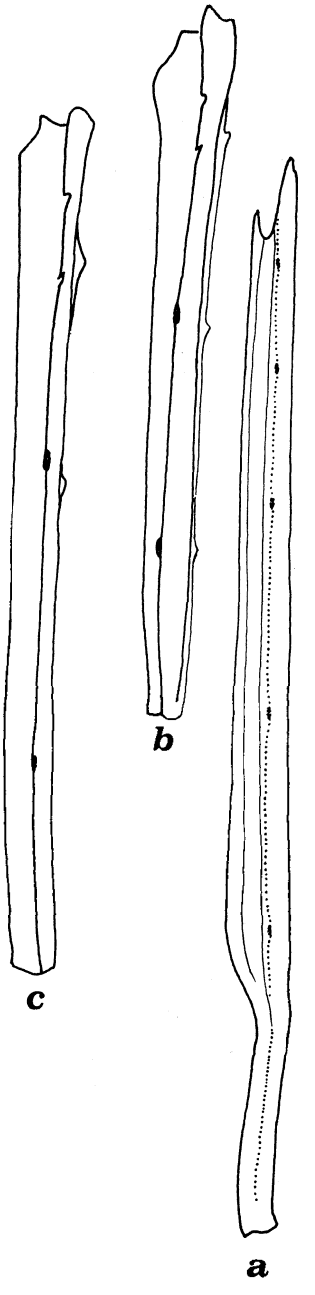

Fig. 4. – *Bacillus lynceorum* from Santo Pietro (Sicily): fore (*a*), median (*b*), and hind (*c*) femora: lateral–ventral view.

3 ♀♀ from the type locality (September-November 1982, L. Bullini and G. Nascetti leg.). Preserved respectively in the collections of the Civic Natural History Museums in Genoa, Milan, and Verona; other material examined: several females collected in the autumn 1982 along the Dirillo and Ficuzza rivers (between Raguse and Caltagirone), near Santo Pietro (Catania district), and near Vittoria (Raguse district); F_1 females obtained in the laboratory.

Diagnostic characters - Differentiated from *B. grandii* and *B. whitei* by the following characters:

- the more developed granulation of meso- and metathorax (Fig. 2);

- the size and shape of lamina subgenitalis, intermediate between those of *B. grandii* and *B. whitei*, with a pronounced notch at its distal edge (Fig. 3);

- the capsule sculpturing and operculum morphology of the egg (Fig. 5, top row);

- the karyotype, allotriploid, with 52 chromosomes (Fig. 6);

- the electrophoretic pattern of phosphoglucose isomerase (*Pgi*).

Holotype description - Adult female; colour brown. Total length 99.5 mm. 19 articles antennae, 8.2 mm long. Head: 4.5 mm long. Thorax segments length: pronotum 4.1 mm, mesonotum 18.6 mm, metanotum + median segment 19.0 mm. Meso- and metathorax with evident granulation. Lamina subgenitalis tapered, with a pronounced notch at its distal edge, reaching about 3/4 of the 9° segment. Cerci: 1.5 mm long. Femora slightly denticulated (Fig. 4); length: fore 27.1 mm, median 17.5 mm, hind 21.3 mm. Tibiae length: fore 28.2 mm, median 15.7 mm, hind 19.8 mm. Legs colour: as in the rest of the body.

Paratypes description - All adult females; colour light brown to brown. Total length: 82.1–115.5 mm. 20–21 articles antennae, 6.4–10.2 mm long. Head: 3.9–5.2 mm long. Thorax segments length: pronotum 3.4–5.0 mm, mesonotum 15.5–19.8 mm, metanotum 16.1–21.2 mm. Meso- and metanotum with evident granulation. Lamina subgenitalis tapered, with a pronounced notch at its distal end, reaching about 3/4 of the 9° segment. Cerci: 1.3–1.6 mm long. Femora slightly denticulated; length: fore 21.7–32.8 mm, median 13.9–21.2 mm, hind 17.2–26.8 mm. Tibiae length: fore 21.8–33.0 mm, median 12.3–18.3 mm, hind 16.1–24.9 mm. Legs colour: as in the rest of the body.

Egg - Colour dark brown. Average length 3.1 mm (range 2.9–3.3 mm); width 2.0 mm (1.9–2.2 mm). Capsule surface with clearly raised ridges (Fig. 5*a*). Micropylar plate covering the whole length of the dorsal plate, limited by two lateral ridges that continue in the operculum rim (Fig. 5*b*). Operculum with

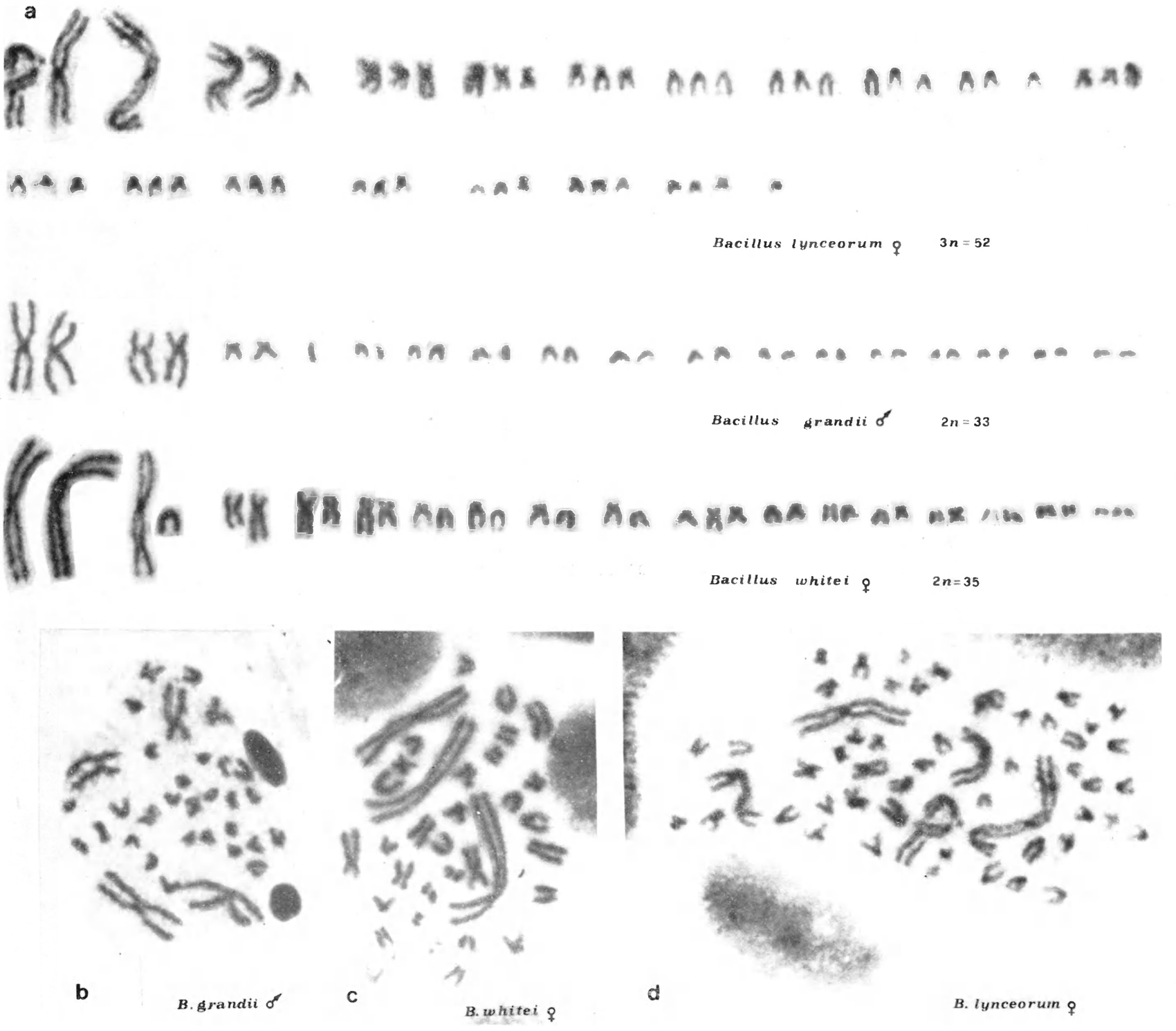

Fig. 6. – *a*: Karyotypes of *Bacillus lynceorum* ♀ (top), *B. grandii* ♂ (centre), and *B. whitei* ♀ (bottom); mitotic metaphase chromosomes, stained with Giemsa, from the follicular epithelium of ovarioles (*B. lynceorum* and *B. whitei*) and from the spermatogonial line (*B. grandii*). *b*, *c*, *d*: mitotic metaphases of *B. grandii* (♂), *B. whitei* (♀), and *B. lynceorum* (♀); staining and tissues are the same as for *a*.

incomplete rim and with an uneven ring inside the rim; irregularly developed cristae converging towards the centre, forming a few chambers (Fig. 5*c*).

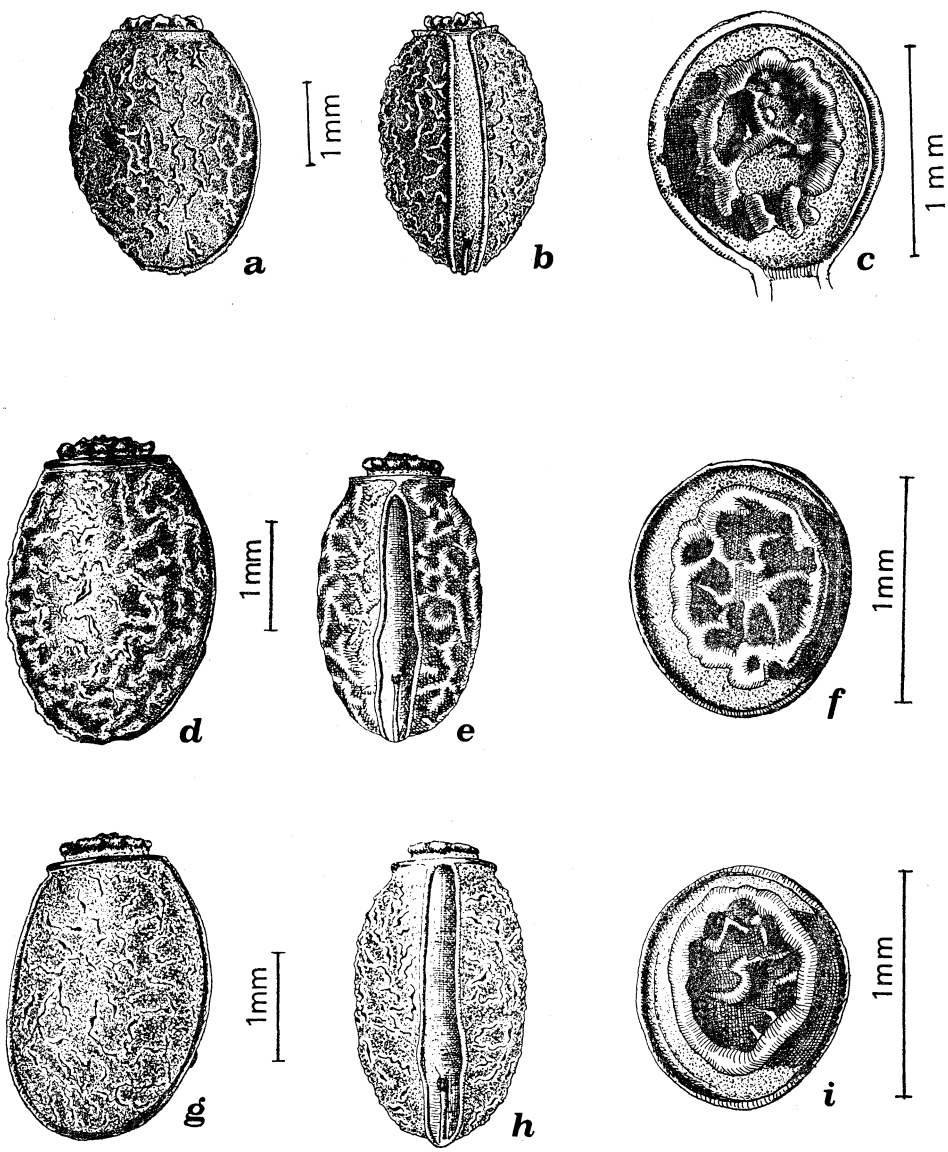

Fig. 5. – Eggs of *Bacillus lynceorum* (top row), *B. grandii* (central row), and *B. whitei* (bottom row); *a*, *d*, *g*: lateral view; *b*, *e*, *h*: dorsal view; *c*, *f*, *i*: operculum, external surface.

Karyotype – ♀: $3n=52$, including 3 large metacentric, 2 large submetacentric, 1 medium metacentric, 10 medium submetacentric, 6 medium subtelocentric, 5 medium telocentric, 5 small metacentric, 7 small submetacentric,

2 small subtelocentric, and 11 small telocentric (Fig. 6). *B. lynceorum* karyotype combines, as shown in Fig. 6, the haploid complement of *B. grandii* ($n = 16 + X$) and the diploid complement of *B. whitei* ($2n = 35$). Accordingly, *B. lynceorum* would be an allotriploid; its origin will be discussed below.

TABLE I

Phenotypes and genotypes of B. lynceorum *at 20 enzyme loci.*

Loci	Observed Phenotypes	Presumed Genotypes
α-Gpdh	*100*	*100/100/100*
Sdh	*106/115*	*106/115/115*
Ldh	*100*	*100/100/100*
Mdh-1	*100/107*	*100/107/107*
Mdh-2	*100/105*	*100/105/105*
Idh-1	*90/100*	*90/100/100*
Idh-2	*92/100*	*92/ 92/100*
6Pgdh	*100/106*	*100/106/106*
G3pdh	*100/115*	*100/115/115*
Hbdh	*82/100*	*82/ 82/100*
Sod	*82/100*	*82/ 82/100*
Got-1	*92/100*	*92/ 92/100*
Got-2	*100*	*100/100/100*
Hk-1	*100/104*	*100/104/104*
Hk-2	*100*	*100/100/100*
Adk	*105*	*105/105/105*
Pgm	*100/105*	*100/105/105*
Ald	*100*	*100/100/100*
Mpi	*100/110*	*100/110/110*
Pgi	*73/85/100*	*73/ 85/100*

Gene structure - The results of the electrophoretic study of 20 enzyme loci (*α-Gpdh*, *Sdh*, *Ldh*, *Hbdh*, *Mdh-1*, *Mdh-2*, *Idh-1*, *Idh-2*, *6Pgdh*, *G3pdh*, *Sod-1*, *Got-1*, *Got-2*, *Hk-1*, *Hk-2*, *Adk*, *Pgm*, *Ald*, *Mpi*, and *Pgi*) are reported in Table I. 14 loci show fixed heterozygosity with two or three (*Pgi*)

distinct electrophoretic alleles; the other 6 loci appear monomorphic. The presumed genotypes at the loci studied are reported. *B. lynceorum* cumulates the alleles previously found in *B. grandii* and in *B. whitei* (see Nascetti and Bullini [6]; Bullini [2]), with only one exception: the allele Pgi^{85}, not previously recorded.

Reproduction – By obligatory thelytokous parthenogenesis; no males recorded up to now.

Distribution and ecology – Found in Southern Sicily (Catania and Raguse districts); lives from 100 to 300 m above sea level, feeding on *Rubus*. Chiefly active during the night. As in *B. grandii* and *B. whitei*, larvae hatch in late spring, from overwintering eggs. Adults generally survive up to the beginning of winter.

Derivatio nominis – The species is named in honour of the Accademia Nazionale dei Lincei, which promoted and supported the research on the Sicilian fauna, during which the species was discovered.

DISCUSSION

The chromosome and electrophoretic evidence reported above clearly indicate a hybrid origin for *B. lynceorum*. This allotriploid parthenogenetic species apparently originated from the cross between *B. grandii* male and *B. whitei* female. The latter species, normally reproducing by parthenogenesis, had itself a hybrid origin, its bisexual ancestors being *B. grandii* and *B. rossius* (Nascetti and Bullini [5]; Nascetti *et al.* [7]; Bullini [2]). The speciation of *B. lynceorum* appears to be a recent event. The genetic structure of its ancestors is quite similar, both at the gene and chromosome levels, to that of today's populations of *B. grandii* and *B. whitei*. The only exception is represented by the Pgi^{85} allele, found in *B. lynceorum* (whose *Pgi* genotype is *73/85/100*) and not recorded up to now in its parental species. The *85* allele was presumably present in the *B. grandii* population involved in the speciation of *B. lynceorum* (the alleles Pgi^{73} and Pgi^{100} are present, in fixed heterozygous condition, in *B. whitei*). It is to be noticed that *B. grandii* is now absent from *B. lynceorum* range, the known populations of the former species living in a few areas of the Syracuse district.

The speciation of *B. lynceorum* (as in the other stick-insects of hybrid origin discovered up to now) did not involve a doubling of the chromosome number, an event frequently occurring in allopolyploid species.

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