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Nothocasis rosariae sp. n., a new sylvicolous, montane species from southern Europe (Lepidoptera: Geometridae, Larentiinae)

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Abstract

In this paper, we describe *Nothocasis rosariae* **sp. n.** as the second European species belonging to the genus *Nothocasis* Prout, 1937. Differential features from its allopatric sibling species *N. sertata* (Hübner, 1817) are presented basing on wing pattern, morphology of male and female genitalia, and molecular data (COI barcode region). The type series is designated from southern Italy, but one examined specimen was collected in Epirus, Greece. The largest phenotypic and genetic variation was observed in the Pollino Massif, northern Calabria, whilst the population of the locus typicus in the Sila Massif, central Calabria, appears to be more homogeneous. 128 individuals were collected in mountainous beech forests from late August to mid-November. We hypothesize that larvae of *N. rosariae* **sp. n.** feed on *Fagus sylvatica* whilst those of its sibling species, *N. sertata*, feed on *Acer*.

Key words: new species, biodiversity, DNA barcode, Mediterranean forests, Italy

Introduction

The genus *Nothocasis* Prout, 1937 (Geometridae: Larentiinae) includes twelve known species mainly distributed in the eastern Palaearctic and northern Indo-Pacific regions (Scoble & Hausmann 2007; Hausmann & Viidalepp 2012). So far, *Nothocasis sertata* (Hübner, 1817) was considered to be the only species occurring in Europe. It has been recorded from central France to the Carpathians and from southernmost Sweden to the Alps. In southern Europe it occurs in very isolated populations in mountainous areas such as the Apennines and mountains of the Balkans. Easternmost isolated populations have been found in southern Ukraine, Caucasus and Transcaucasus (Hausmann & Viidalepp 2012).

Few synonymic names are existing in literature for *Nothocasis sertata* due to the putative absence of closely related species:

Lobophora sertata var. f. *fumidata* Turati & Verity, 1912 (Maritime Alps: Terme [di Valdieri], Italy);
Lobophora sertata f. *tangens* Wehrli, 1917 (Jura: Aargau and Basel, Switzerland) [infrasubspecific];
Acasis sertata f. *viridulata* Turati, 1919 (Northern Apennines (province of Modena): Sestola, 1000 m, Italy);
Lobophora sertata ab. *dissoluta* Höfer, 1920 (Lower Austria) [infrasubspecific];
Lobophora sertata ab. *costimaculata* Höfer, 1920 (Lower Austria) [infrasubspecific];
Lobophora sertata ab. *neofasciata* Höfer, 1920 (Lower Austria) [infrasubspecific];
Lobophora sertata var. *nigrofasciata* Osthelder, 1929 (Bavaria: Allgäuer Alps, Germany). This form is identical to *neofasciata* Höfer, 1920 according to Prout (1936).
Lobophora sertata var. *obscurata* Osthelder, 1929 (Bavaria: Allgäuer Alps, Germany);
Lobophora sertata ab. (?) *hilariata* Dannehl, 1933 (Black Forest: Pforzheim, Germany) [infrasubspecific?—The author writes: "Derartige Falter erhielt ich wiederholt und in Anzahl aus dem Schwarzwald, wo sich vielleicht eine distinkte Rasse gebildet hat oder in der Bildung begriffen ist"].

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Doubts about the identity of isolated populations of *Nothocasis sertata* in southern Europe arose thanks to DNA barcoding analyses. Hausmann & Viidalepp (2012) found a great homogeneity in the mitochondrial gene COI 5' from Germany to Croatia, but noted a divergence of 2.4% from a specimen from Greece. Recent research on the lepidopteran fauna of Calabria, the southernmost region of peninsular Italy, led to the discovery of new populations at a first glance belonging to *Nothocasis sertata*. Morphological and genetic studies confirmed the presence of an undescribed species which is closely related but clearly distinguishable from *N. sertata* in Calabria.

Material and methods

Material of the type series was collected by using various types of light traps in a few localities of the Sila and Pollino Massifs (see below, under material examined). We used a Rothamsted light trap with a 200W incandescence lamp for moth collecting in the type locality of Vivaio Sbanditi, Sila Massif, whilst in the other localities we used Head traps equipped with 12W UV LEDs. Examined material is stored in the following collections: Zoologische Staatssammlung München (SNSB-ZSM), Germany; Collection of the Unità di Ricerca per la Selvicoltura in Ambiente Mediterraneo (Crea-Sam), Italy.

Species delimitation was based on the combined study of adults (coloration, pattern, external morphology) and morphology of genitalia, as well as genetics. Genitalia extraction and slide mounting were performed following standard methodology (Parenti 2000). Terminology of genitalia traits follows Hausmann (2001). Genetic analyses were carried out on the mitochondrial cytochrome c oxidase I (COI) gene (658 base pairs), the standard DNA fragment used in animals to help taxonomists in species identification and delimitation (Hajibabaei *et al*. 2006, Hausmann *et al*. 2013; Miller *et al*. 2016). One leg was detached from specimens and submitted to standard protocols for DNA barcoding analysis (Ivanova *et al*. 2006; CCDB, 2015), carried out in the Canadian Centre for DNA Barcoding, Guelph, Ontario, Canada. LepF1 and LepR1 were the primers used for PCR and sequencing (Hajibabaei *et al*. 2006). Inter- and intra-specific genetic variations were calculated using the Kimura 2-parameter model (Kimura 1980) and the neighbour-joining algorithm (Saitou & Nei 1987), as implemented in BOLD (http://www.boldsystems.org/). All recovered sequences of the new species were 658 bp long, and were assigned to the Barcode Index Number (BIN) BOLD:AAM2755. Sequences and metadata are accessible for public in the BOLD-dataset DS-NOTHOCAS. Data about analyzed sequences are summarized in Table 1.

Results

Nothocasis rosariae Scalercio, Infusino & Hausmann, new species

Material examined. Holotype male: HOLOTYPE / Nothocasis / rosariae [red rectangle label]; Italia Calabria / Sila—Vivaio Sbanditi (CS) / 1350m—8.9.15 / 39.3889°, 16.6022° / leg. Scalercio & Infusino (Coll. Crea-Sam, Italy). Paratypes altogether 21 males and 16 females. 1 male: Italia Calabria / Sila—Vivaio Sbanditi (CS) / 1350m—22/09/2014 / 39°23'20"N; 16°36'08"E / leg. S. Scalercio; Prep. number CREASAM 42, Stefano Scalercio [DNA barcode specimen ID LEP-SS-00294]. 1 male: Italia Calabria / Sila—Vivaio Sbanditi (CS) / 1350m—13/10/2014 / 39°23'20"N; 16°36'08"E / leg. S. Scalercio; Prep. number CREASAM 50, Stefano Scalercio [DNA barcode specimen ID LEP-SS-00162]. 1 female: Italia Calabria / Sila—Vivaio Sbanditi (CS) / 1350m—13/10/2014 / 39°23'20"N; 16°36'08"E / leg. S. Scalercio; Prep. number CREASAM 58, Stefano Scalercio [DNA barcode specimen ID LEP-SS-00295]. 1 female: Italia Calabria / Sila—Vivaio Sbanditi (CS) / 1350m—13/10/2014 / 39°23'20"N; 16°36'08"E / leg. S. Scalercio; Prep. number CREASAM 51, Stefano Scalercio [DNA barcode specimen ID LEP-SS-00163]. 3 males: Italia Calabria / Sila—Vivaio Sbanditi (CS) / 1356m—20.x.15 / 39.3889°, 16.6022° / leg. Scalercio & Infusino. 1 male: Italia Calabria / Sila—Vivaio Sbanditi (CS) / 1356m—15.ix.15 / 39.3889°, 16.6022° / leg. Scalercio & Infusino. 1 female: Italia Calabria—SL_A1 / Sila, Montagna Grande (CS) / 1370 m—05/10/2015 / 39.2766°, 16.6102° / leg. Scalercio & Infusino; Prep. number CREASAM 59, Stefano Scalercio. 1 male: Italia Calabria—PO_A1 / Serrapaolo, Saracena (CS) / 990 m—24/08/2015 / 39.8225°, 16.0911° / leg. Scalercio & Infusino; Prep. number CREASAM 57, Stefano Scalercio. 1 female:

Italia Calabria—PO_A1 / Serrapaolo, Saracena (CS) / 990 m—14/10/2015 / 39.8225°, 16.0911° / leg. Scalercio & Infusino. 2 females: Italia Calabria—PO_A1 / Serrapaolo, Saracena (CS) / 990 m—18.xi.2015 / 39.8225°, 16.0911° / leg. Scalercio & Infusino. 1 male: Italia Calabria—PO_A2 / Serrapaolo, Saracena (CS) / 1010 m—24/08/2015 / 39.8225°, 16.0883° / leg. Scalercio & Infusino [DNA barcode specimen ID LEP-SS-00364]. 1 male: Italia Calabria—PO_B2 / Bruscata, Saracena (CS) / 1370 m—24/08/2015 / 39.8103°, 16.0468° / leg. Scalercio & Infusino [DNA barcode specimen ID LEP-SS-00363]. 1 male and 1 female: Italia Calabria—PO_B2 / Bruscata, Saracena (CS) / 1370 m—14/10/2015 / 39.8103°, 16.0468° / leg. Scalercio & Infusino. 1 female: Italia Calabria—PO_C1 / T.ne Magara, Saracena (CS) / 1465 m—18.xi.15 / 39.7939°, 16.0520° / leg. Scalercio & Infusino. 1 male: Italia Calabria—PO_C2 / T.ne Magara, Saracena (CS) / 1460 m—24/08/2015 / 39.7914°, 16.0503° / leg. Scalercio & Infusino [DNA barcode specimen ID LEP-SS-00361]. 1 male: Italia Calabria—PO_C2 / T.ne Magara, Saracena (CS) / 1460 m—24/08/2015 / 39.7914°, 16.0503° / leg. Scalercio & Infusino [DNA barcode specimen ID LEP-SS-00362]. 1 female: Italia Calabria—PO_C2 / T.ne Magara, Saracena (CS) / 1460 m—24/08/2015 / 39.7914°, 16.0503° / leg. Scalercio & Infusino [DNA barcode specimen ID LEP-SS-00360]. 1 female: Italia Calabria—PO_C2 / T.ne Magara, Saracena (CS) / 1460 m—14/10/2015 / 39.7914°, 16.0503° / leg. Scalercio & Infusino. 1 male and 1 female: Italia Calabria—PO_C2 / T.ne Magara, Saracena (CS) / 1460 m—14/10/2015 / 39.7914°, 16.0503° / leg. Scalercio & Infusino. 1 female: Italia Calabria—PO_C2 / T.ne Magara, Saracena (CS) / 1460 m—18.xi.15 / 39.7914°, 16.0503° / leg. Scalercio & Infusino; Prep. number CREASAM 60, Stefano Scalercio. 1 male: Italia Calabria—PO_C3 / T.ne Magara, Saracena (CS) / 1475 m—24/08/2015 / 39.7859°, 16.0523° / leg. Scalercio & Infusino. 1 male: Italia Calabria—PO_C3 / T.ne Magara, Saracena (CS) / 1475 m—14/10/2015 / 39.7859°, 16.0523° / leg. Scalercio & Infusino (all in Coll. Crea-Sam). 1 female: Italia Calabria / Sila—Vivaio Sbanditi (CS) / 1350m—6.x.15 / 39.3889°, 16.6022° / leg. Scalercio & Infusino. 2 males: Italia Calabria—PO_A1 / Serrapaolo, Saracena (CS) / 990 m—14/10/2015 / 39.8225°, 16.0911° / leg. Scalercio & Infusino. 1 male: Italia Calabria—PO_A2 / Serrapaolo, Saracena (CS) / 1010 m—14/10/2015 / 39.8225°, 16.0883° / leg. Scalercio & Infusino. 1 male: Italia Calabria—PO_B2 / Bruscata, Saracena (CS) / 1370 m—24/08/2015 / 39.8103°, 16.0468° / leg. Scalercio & Infusino. 2 females: Italia Calabria—PO_B2 / Bruscata, Saracena (CS) / 1370 m—14/10/2015 / 39.8103°, 16.0468° / leg. Scalercio & Infusino. 1 female: Italia Calabria—PO_C1 / T.ne Magara, Saracena (CS) / 1465 m—18.xi.15 / 39.7939°, 16.0520° / leg. Scalercio & Infusino. 2 males: Italia Calabria—PO_C2 / T.ne Magara, Saracena (CS) / 1460 m—14/10/2015 / 39.7914°, 16.0503° / leg. Scalercio & Infusino (all in coll. ZSM).

Other material examined. 4 males and 3 females: Serrapaolo (PO_A1), Saracena (CS), Italy, 990 m, 14.x.2015, 39.8225°, 16.0911°, leg. Scalercio & Infusino. 6 males and 9 females: Bruscata (PO_B2), Saracena (CS), Italy, 1370 m, 14.x.2015, 39.8103°, 16.0468°, leg. Scalercio & Infusino. 2 males: Bocca di Novacco (PO_B3), Saracena (CS), Italy, 1340 m, 14.x.2015, 39.8140°, 16.0451°, leg. Scalercio & Infusino. 4 males and 1 female: Timpone Magara (PO_C1), Saracena (CS), Italy, 1465 m, 23.ix.2015, 39.7939°, 16.0520°, leg. Scalercio & Infusino. 5 males and 6 females: Timpone Magara (PO_C1), Saracena (CS), Italy, 1465 m, 14.x.2015, 39.7939°, 16.0520°, leg. Scalercio & Infusino. 4 males and 3 females: T.ne Magara (PO_C2), Saracena (CS), Italy, 1460 m, 23.ix.2015, 39.7914°, 16.0503°, leg. Scalercio & Infusino. 20 males and 24 females: T.ne Magara (PO_C2), Saracena (CS), Italy, 1460 m, 14.x.2015, 39.7914°, 16.0503°, leg. Scalercio & Infusino. 1 male: Greece, Epirus, 20km N Konitza, 40.1906 N, 20.7966 E, 20-Oct-2009, 520m, leg. Dr. Grünewald [DNA barcode specimen ID BC ZSM Lep 35649]. 1 male: Italy, Basilicata, Monte Sirino W, forestry road, 1570m, 40.2417° N, 16.3633° E, 05-Sep-2011, leg. A. Hausmann [DNA barcode specimen ID BC ZSM Lep 60930].

Description. External characters (Figs. 1–4): Wingspan male 22-28 mm (n=17), 26 mm for the holotype (Fig. 1), wingspan female 23–29 mm (n=21). Wings light grey. Transverse lines of forewings parallel to distal margin (termen) of the wing, black or dark grey, bordered by brownish scales especially along basal, medial and post-medial lines. Terminal line dissolved to a row of paired black dots, one above and the other below distal terminations of veins. Discal spots black, surrounded by a pale ring. Forewing pattern, especially in male, not well contrasted and defined due to irroration by dark grey scales. Fringe concolorous with wings, sometimes darker at vein terminations. Hindwing without transverse lines, with terminal area slightly darker. Discal spots black. Underside of wings as upperside, but paler. Frons, thorax and abdomen concolorous with wings. Antennae ciliate-setose in males and scarcely ciliate in females, flagellum dorsally chequered black and white. Hindtibia of both sexes with one pair of distal spurs, male hindtibia with a pencil longer than the tibia. Tympanal organs medium-sized.

FIGURE 1. *Nothocasis rosariae* **sp. n.**, male, 8.ix.2015, Vivaio Sbanditi, Calabria, Italy (Coll. Crea-Sam), holotype.

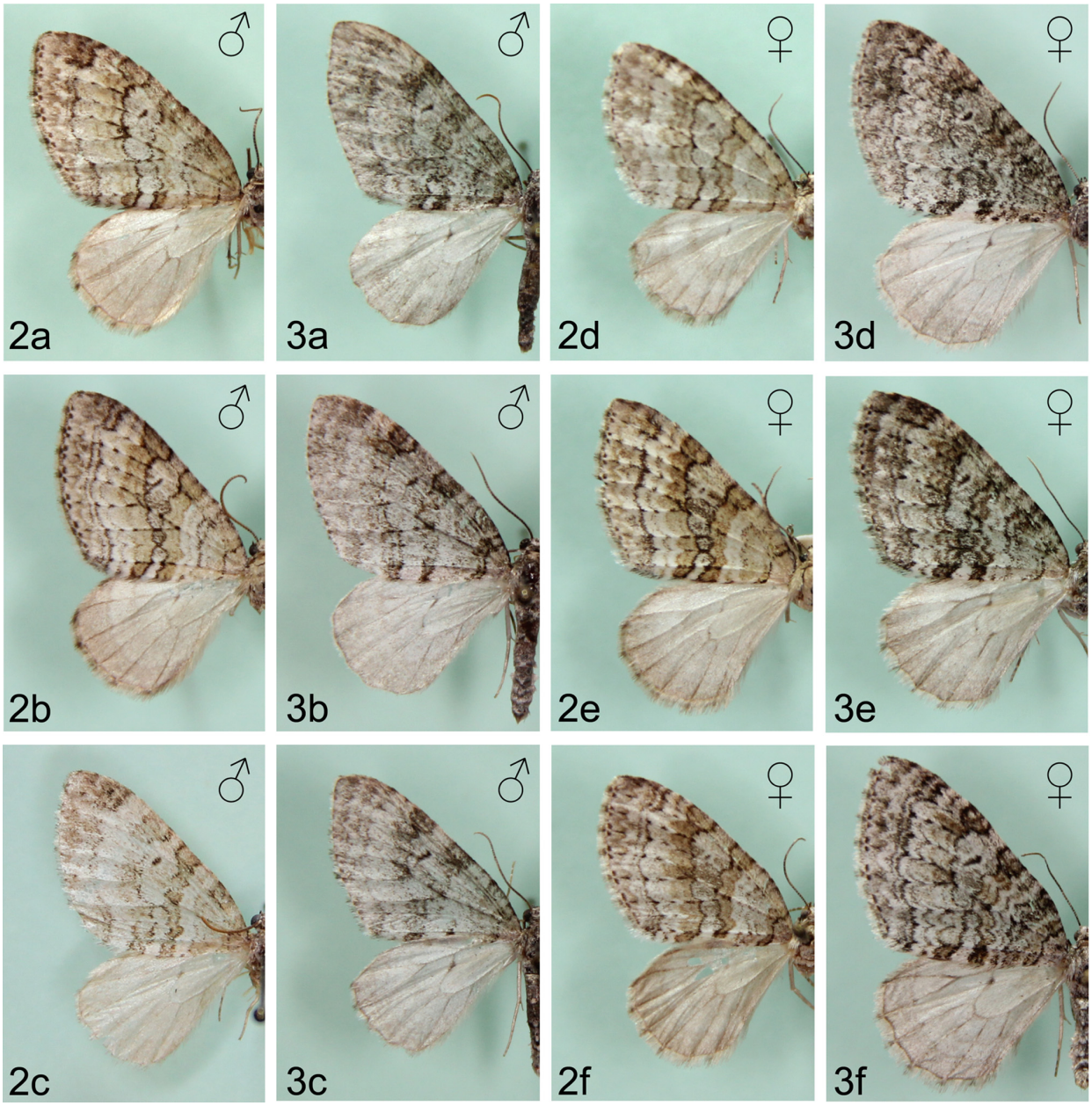
Male genitalia (Figs. 5–12): Uncus long, tubular, distal portion sparsely setose, width constant from the basis to the tip, inversely T-shaped. Socii and gnathos absent. Valva setose, bilobed at tip. Apices of the cleft valva densely setose. Costa strongly sclerotized. Harpe well developed, forked, V-shaped distally, with two pointed tips. Juxta large, trapezoidal. Saccus short and flat. Aedeagus short, narrow at base and at tip, slightly dilated at 4/5 of its length. Vesica without spinose cornuti.

Female genitalia (Figs. 13–16): Papillae anales wide, setose. Apophyses posteriores long, slightly curved at 1/5 of their length. Apophyses anteriores more robust, 1/4 length of apophyses posteriores. Antrum well sclerotized, cup-shaped, showing in mounted genitalia a width/length ratio 3.3–3.6 (n=3) and bidimensional shape with apices pointed and widely projecting laterally. Ductus bursae short, not sclerotized. Ductus seminalis projecting from short membranous appendix bursae between ductus bursae and corpus bursae. Posterior part of corpus bursae narrowly cylindrical, weakly sclerotized, slightly granulated or with sparse microspines. Anterior part of corpus bursae elliptic, membranous, without signum.

Variation (Fig. 4). Forewing pattern more variable than hindwing pattern. Forewing sometimes with two anastomoses of medial and postmedial lines on veins CuA1 and CuA2. Forewing ground colour varying from dark grey to light grey. Some specimens with a dark grey medial fascia, bordered by brownish antemedial and postmedial fasciae. Such variants have been observed throughout the entire flight season and in both sexes, but only in the populations of Pollino Massif, while the wing pattern in the populations of the Sila Massif were much more homogeneous.

Genetic data (Fig. 17). Genetically heterogeneous in the distribution area (Italy and Greece), mean intraspecific variation 0.65%, maximum variation 1.93% (n=11). Nearest species: *Nothocasis sertata* (minimum pairwise distance 2.25%). Within the known range of *N. rosariae* it is possible to identify three genetic lineages consistent with different geographic areas. Populations of peninsular Italy are grouped in two different clusters at a minimum pairwise distance of 0.50% (=three basepairs). One cluster refers to specimens from the Pollino Massif (maximum intra-populational variation 0.23%) and the other to specimens from the Sila Massif (maximum intra-populational variation 0%). The single specimen from Epirus, Greece, diverges by 1.77% (minimum pairwise

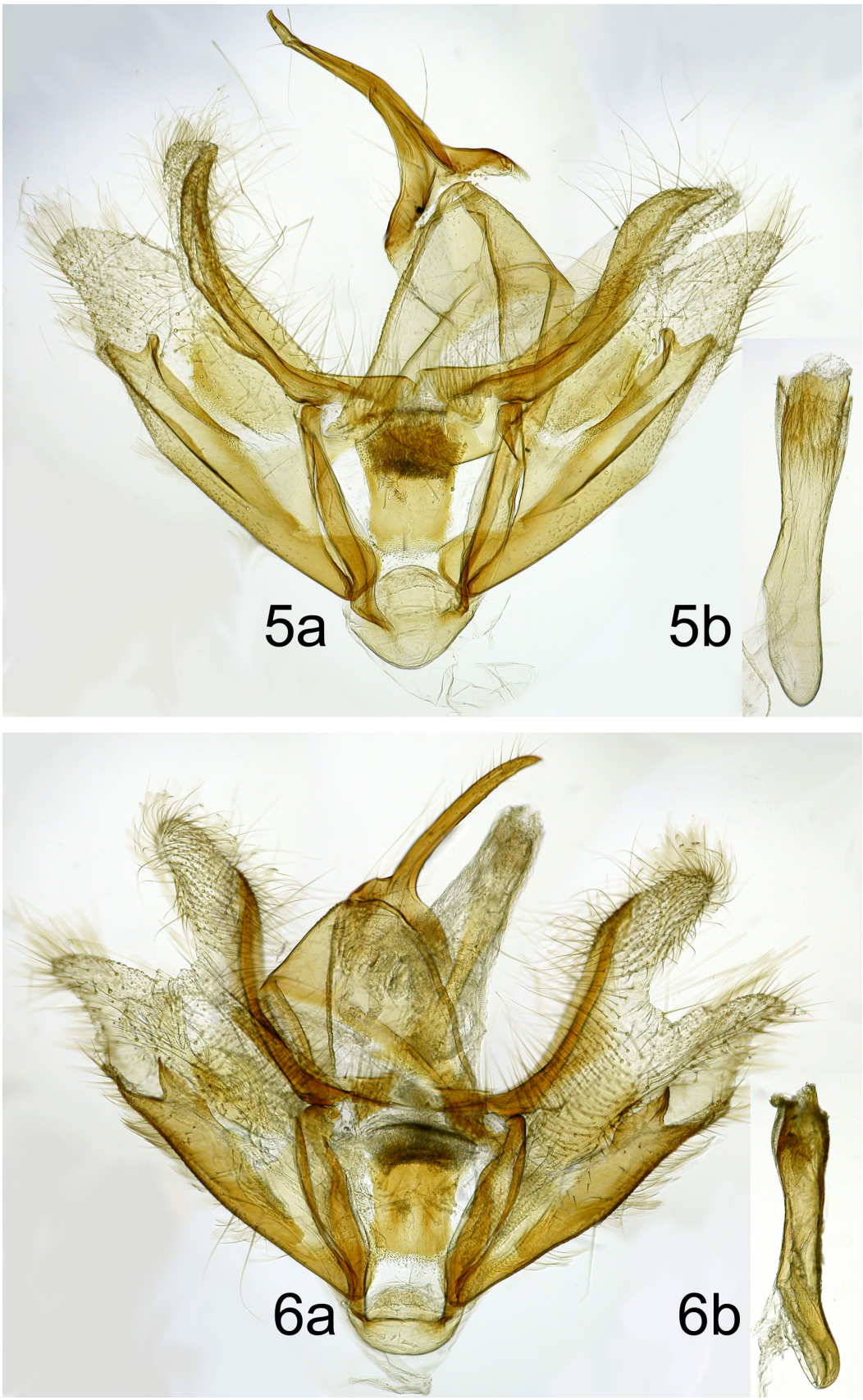
distance) from the Italian populations, by 2.85% from *N. sertata*. So far, no other specimens of *Nothocasis* have been sequenced to the full barcode fragment (658bp), but a short fragment (307bp) of *Nothocasis polystictaria* from Nepal at a genetic distance of 14.1% (Kimura 2, complete deletion) from the European species seems to question its taxonomic position within the genus. The same applies for two short sequences (164bp) of the holotypes of *N. posteropuncta* and *N. hyberniata*, both described from Nepal and both at genetic distances of 10.8%.



FIGURES 2–3. Comparison of *Nothocasis sertata* (Hübner) and *N. rosariae* **sp. n.** 2a. ***Nothocasis sertata*** (Hübner), male, 10.ix.1925, Ampflwang, Oberösterreich, Austria (Coll. ZSM). 2b. *N. sertata*, male, 24.ix.1925, Kelheim, Südbayern, Germany (Coll. ZSM). 2c. *N. sertata*, male, 23.ix.1997, Prato alle Cogne, Arezzo, Italy (Coll. Crea-Sam, ex coll. L. Dapporto). 2d. *N. sertata*, female, 21.ix.1924, Northeim, Hannover, Germany (Coll. ZSM). 2e. *N. sertata*, female, 15.vii.1920, Kelheim, Südbayern, Germany (Coll. ZSM). 2f. *N. sertata*, female, 14.ix.1924, Urwald, Saxony, Germany (Coll. ZSM). 3a. ***Nothocasis rosariae*** **sp. n.**, male, 20.x.2015, Vivaio Sbanditi, Longobucco, Calabria, Italy (Coll. Crea-Sam), paratype. 3b. *N. rosariae*, male, 24.viii.2015, Bruscata, Saracena, Calabria, Italy (Coll. Crea-Sam), paratype. 3c. *N. rosariae*, male, 14.x.2015, Serrapaolo, Saracena, Calabria, Italy (Coll. Crea-Sam), paratype. 3d. *N. rosariae*, female, 5.x.2015, Montagna Grande, San Giovanni in Fiore, Calabria, Italy (Coll. Crea-Sam), paratype. 3e. *N. rosariae*, female, 13.x.2014, Vivaio Sbanditi, Longobucco, Calabria, Italy (Coll. Crea-Sam), paratype. 3f. *N. rosariae*, female, 24.viii.2015, Timpone Magara, Saracena, Calabria, Italy (Coll. Crea-Sam), paratype.♀

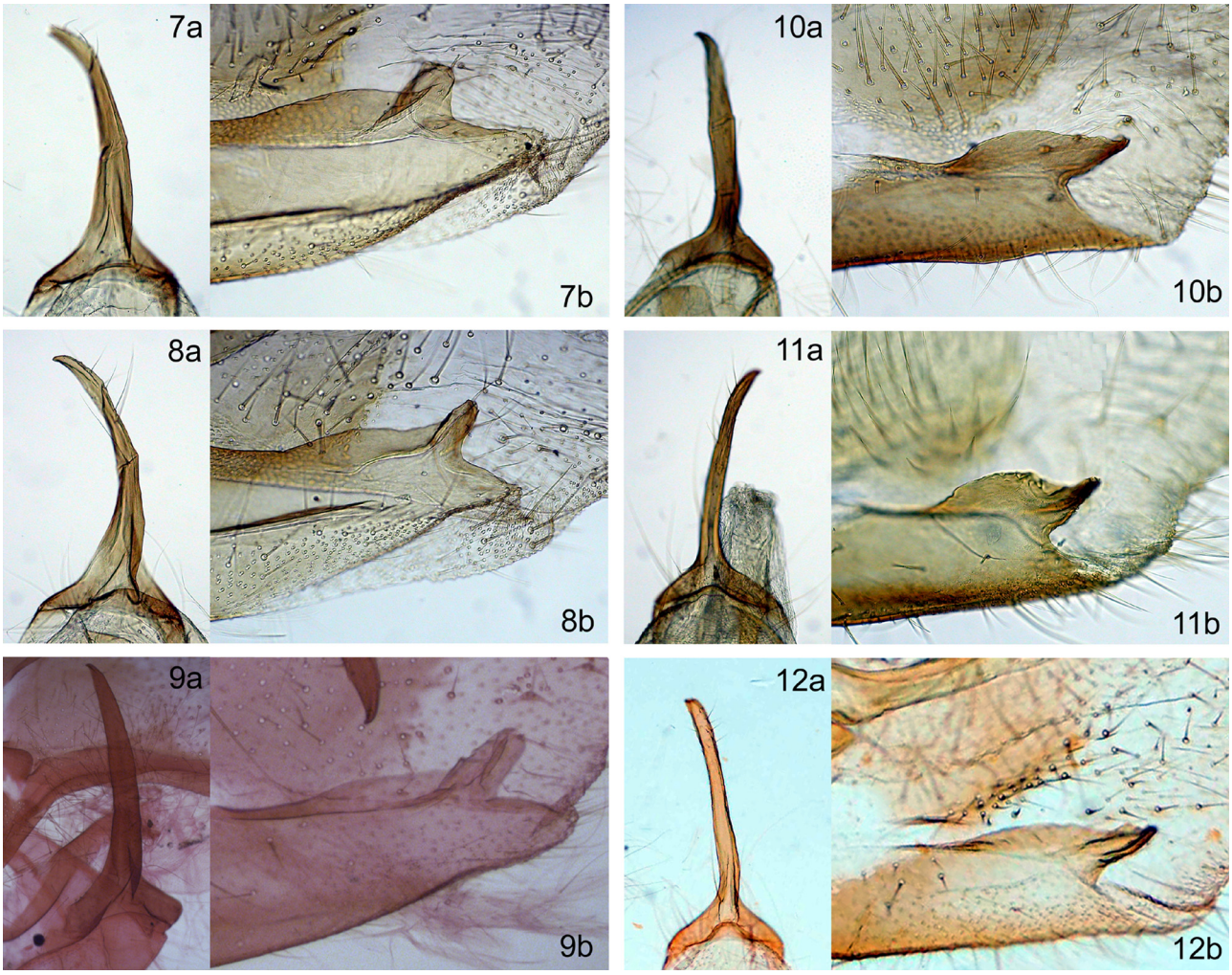
FIGURE 4. Intraspecific variation of adult habitus in *Nothocasis rosariae* **sp. n.**. **a** male, 24.viii.2015, Timpone Magara, Saracena, Calabria, Italy (Coll. Crea-Sam), paratype. **b** male, 24.viii.2015, Timpone Magara, Saracena, Calabria, Italy (Coll. Crea-Sam), paratype. **c** female, 18.xi.2015, Serrapaolo, Saracena, Calabria, Italy (Coll. Crea-Sam), paratype. **d** female, 13.x.2014, Vivaio Sbanditi, Longobucco, Calabria, Italy (Coll. Crea-Sam), paratype. **e** male, 14.x.2015, Timpone Magara, Saracena, Calabria, Italy (Coll. Crea-Sam), paratype. **f** male, 24.viii.2015, Serrapaolo, Saracena, Calabria, Italy (Coll. Crea-Sam), paratype. **g** female, 24.viii.2015, Timpone Magara, Saracena, Calabria, Italy (Coll. Crea-Sam), paratype. **h** female, 14.x.2015, Timpone Magara, Saracena, Calabria, Italy (Coll. Crea-Sam), paratype. **i** male, 14.x.2015, Timpone Magara, Saracena, Calabria, Italy (Coll. Crea-Sam), paratype. **j** male, 14.x.2015, Serrapaolo, Saracena, Calabria, Italy (Coll. Crea-Sam), paratype. **k** female, 14.x.2015, Timpone Magara, Saracena, Calabria, Italy (Coll. Crea-Sam), paratype. **l** 14.X.2015, Bruscata, Saracena, Calabria, Italy (Coll. Crea-Sam), paratype.

Similar species. The new species can be confused with small individuals of *Epirrita* which fly synchronously in the same habitats, and with *Trichopteryx carpinata* (however, with an early spring phenology), both easily distinguishable by dissection of genitalia. Differing from *Nothocasis sertata* in wing pattern (Figs. 2–3), and morphology of genitalia (males: Figs. 5–6, 7–12; females: Figs. 13, 15–16). Most important diagnostic features are:



FIGURES 5–6. Male genitalia (aedeagus separated) of *Nothocasis sertata* (Hübner) and *Nothocasis rosariae* **sp. n.**. **5** *N. sertata*, prep. number ZSM 3, Stefano Scalercio, 16.ix.1922, Northeim, Hannover, Germany (Coll. ZSM), **5a:** genitalia; **5b:** aedeagus. **6** *N. rosariae* **sp. n.**, prep. number CREASAM 50, 13.x.2014, Vivaio Sbanditi, Longobucco, Italy (Coll. Crea-Sam), **6a:** genitalia; **6b:** aedeagus.

- forewing pattern more diffuse than in *N. sertata*, brown scales bordering medial and postmedial lines not arranged in well-defined fasciae;
- uncus tubular, with constant width from basis to tip and inversely T-shaped in *N. rosariae* **sp. n.**, uncus wider at basis and inversely Y-shaped in *N. sertata*;
- Harpe distally with two pointed tips in *N. rosariae* **sp. n.**, bilobous in *N. sertata*;
- width/length ratio of antrum 3.3–3.6 (n=3), with a bidimensional shape in mounted genitalia laterally pointed and widely projecting in *N. rosariae* **sp. n.**, 2.2–2.5 (n=3), with a less diverging and less pointed shape in *N. sertata*;
- Posterior part of corpus bursae narrow and weakly sclerotized, cylindrical in *N. rosariae* **sp. n.**, weakly sclerotized on the entire surface of ductus bursae in *N. sertata*.



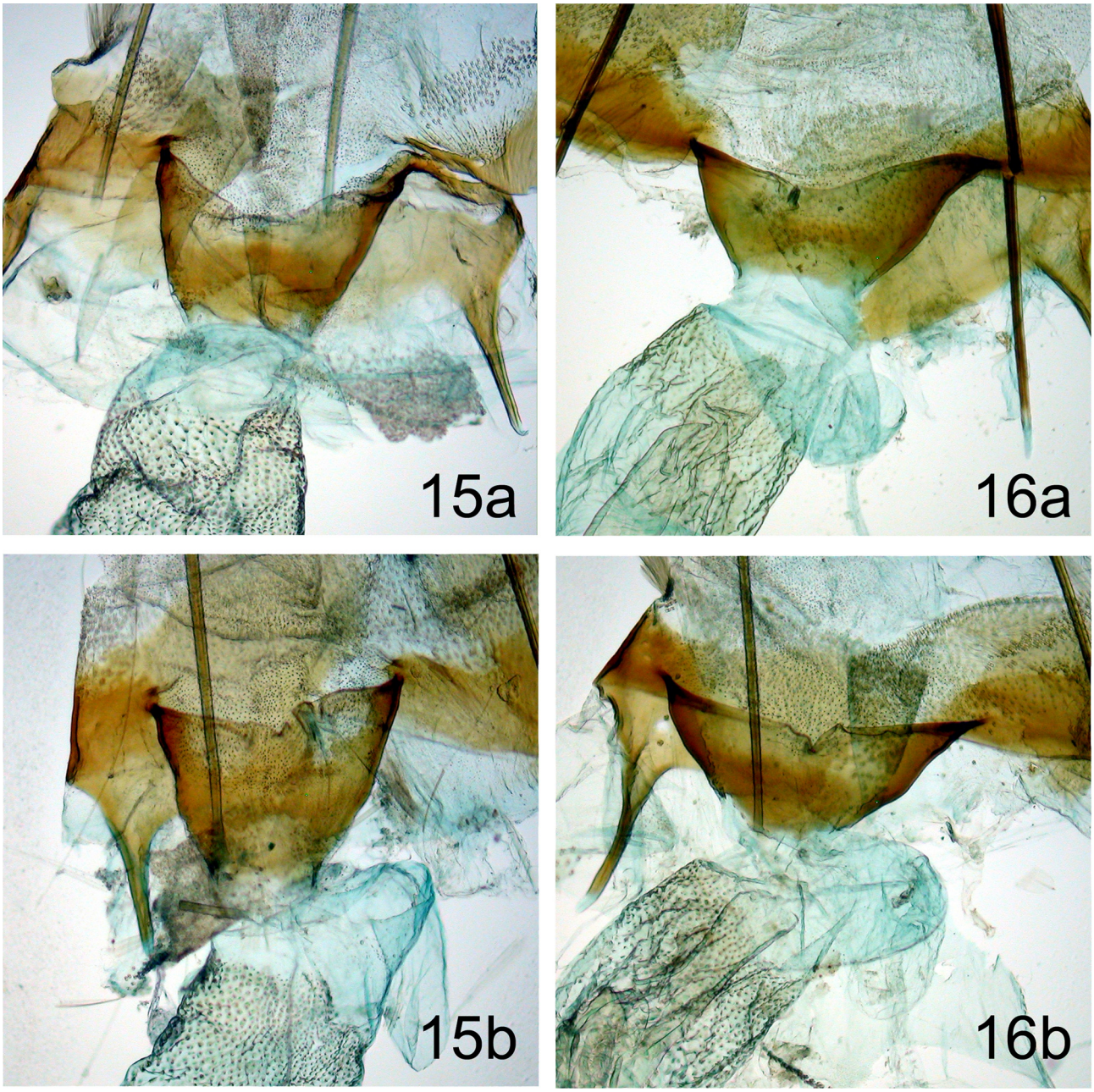
FIGURES 7–12. Uncus and harpe of *Nothocasis sertata* (Hübner) and *Nothocasis rosariae* **sp. n.**. **7a–b** *N. sertata*, prep. number ZSM 1, Stefano Scalercio, 10.ix.1925, Ampflwang., Oberösterreich, Austria (Coll. ZSM). **8a–b** *N. sertata*, prep. number ZSM 2, Stefano Scalercio, 24.ix.1925, Kelheim, Südbayern, Germany (Coll. ZSM). **9a–b** *N. sertata*, prep. number 679, Leonardo Dapporto, 23.ix.1997, Prato alle Cogne, Arezzo, Italy (Coll. L. Dapporto). **10a–b** *N. rosariae*, prep. number CREASAM 57, 24.viii.2015, Serrapaolo, Saracena, Italy (Coll. Crea-Sam). **11a–b** *N. rosariae*, prep. number CREASAM 42, 22.ix.2014, Vivaio Sbanditi, Longobucco, Italy (Coll. Crea-Sam). **12a–b** *N. rosariae*, Greece (Coll. ZSM).

Phenology. During field research we collected 128 individuals belonging to *Nothocasis rosariae*, most of which not designed as paratypes because not mounted with pins. The species starts to fly in late August when few individuals (n=8) have been collected in the beech forest of the Pollino National Park. In September the abundance slightly increased (n=16), reaching its peak in October (n=100). Last individuals were collected as late as mid-November (n=5). Males were much more abundant at the beginning of the flight season, while in November only females were collected.

FIGURES 13–14. Female genitalia of *Nothocasis sertata* (Hübner) and *Nothocasis rosariae* **sp. n.**. **13** *N. sertata*, prep. number ZSM 6, Stefano Scalercio, 21.ix.1924, Northeim, Hannover, Germany (Coll. ZSM). **14** *N. rosariae*, prep. number CREASAM 60, 18.xi.2015, Timpone Magara, Saracena, Italy (Coll. Crea-Sam).

Biology. Unknown. Adults were most abundantly collected within pure *Fagus sylvatica* forests. Without further evidence, we can only hypothesize that larvae most likely feed on beech rather than on *Acer pseudoplatanus*, as its European sibling species *N. sertata* (Hausmann & Viidalepp 2012).

Distribution (Fig. 18). The type specimens have been collected in two localities of the Sila Massif and in three localities of the Pollino Massif (Calabria, Italy), where other ten specimens were collected in two different localities. More abundant populations were found in the Pollino Massif (Calabria, Italy) (n=118). The northernmost population was found on the Mount Sirino (n=1) (Basilicata, Italy). One specimen has been collected in the Epirus (Greece). To date it appears to be a trans-Ionian species, but further investigation is needed to delimitate the range of this species in Italy and in the Balkan countries. One record from Tuscany was confirmed as *N. sertata* through dissection, another from Croatia at the border to Bosnia through DNA barcoding (Iva Mihoci, pers. comm.).



FIGURES 15–16. Details of antrum and adjacent structures. **15a** *Nothocasis sertata* (Hübner), prep. number ZSM 6, Stefano Scalercio, 21.ix.1924, Northeim, Hannover, Germany (Coll. ZSM). **15b** *N. sertata*, prep. number ZSM 4, Stefano Scalercio, 14.ix.1924, Urwald, Saxony, Germany (Coll. ZSM). **16a** *Nothocasis rosariae* **sp. n.**, prep. number CREASAM 60, 18.xi.2015, Timpone Magara, Saracena, Italy (Coll. Crea-Sam). **16b** *N. rosariae* **sp. n.**, prep. number CREASAM 59, 05.x.2015, Montagna Grande, San Giovanni in Fiore, Italy (Coll. Crea-Sam).

Habitat (Figs. 19–20). Forested habitats, especially in beech forests (*Fagus sylvatica*) but found also in Calabrian black pine forests (*Pinus nigra laricio*). Young forests having a closed canopy seems to be preferred to old forests having discontinuity in the canopy cover. Vertical distribution from 520m up to 1475m.

Derivatio nominis. The species is dedicated to Rosaria Calcagnile, the mother of the first author.

Discussion and conclusions

The discovery of a new species of *Nothocasis, N. rosariae* **sp. n.**, in southern Europe questions the correct species identification of known isolated populations of *Nothocasis sertata* in that geographic area. Future studies will

likely enlarge the range of known distribution for *N. rosariae* **sp. n.** in the Balkan countries and Apennine mountains. In northern Italy *Nothocasis sertata* was recorded for several localities, but shows a much more scattered distribution along the Apennines (Parenzan & Porcelli 2006), where it was reported for Emilia (Turati 1919; Flamigni & Bastia 2009), Romagna (Zangheri 1965; Fiumi & Camporesi 1988), Tuscany (Marini & Trentini 1984; Dapporto *et al.* 2005), Umbria (Prola & Racheli 1979), Marche (Teobaldelli 1976, 1978, 2009), Abruzzo (Sciarretta & Zahm 2002). Alpine populations of *Nothocasis* need to be attributed to *N. sertata* as confirmed by barcoding analysis (Fig. 17), and also for Tuscany, the dissection of a male specimen could confirm its identity as *N. sertata*.

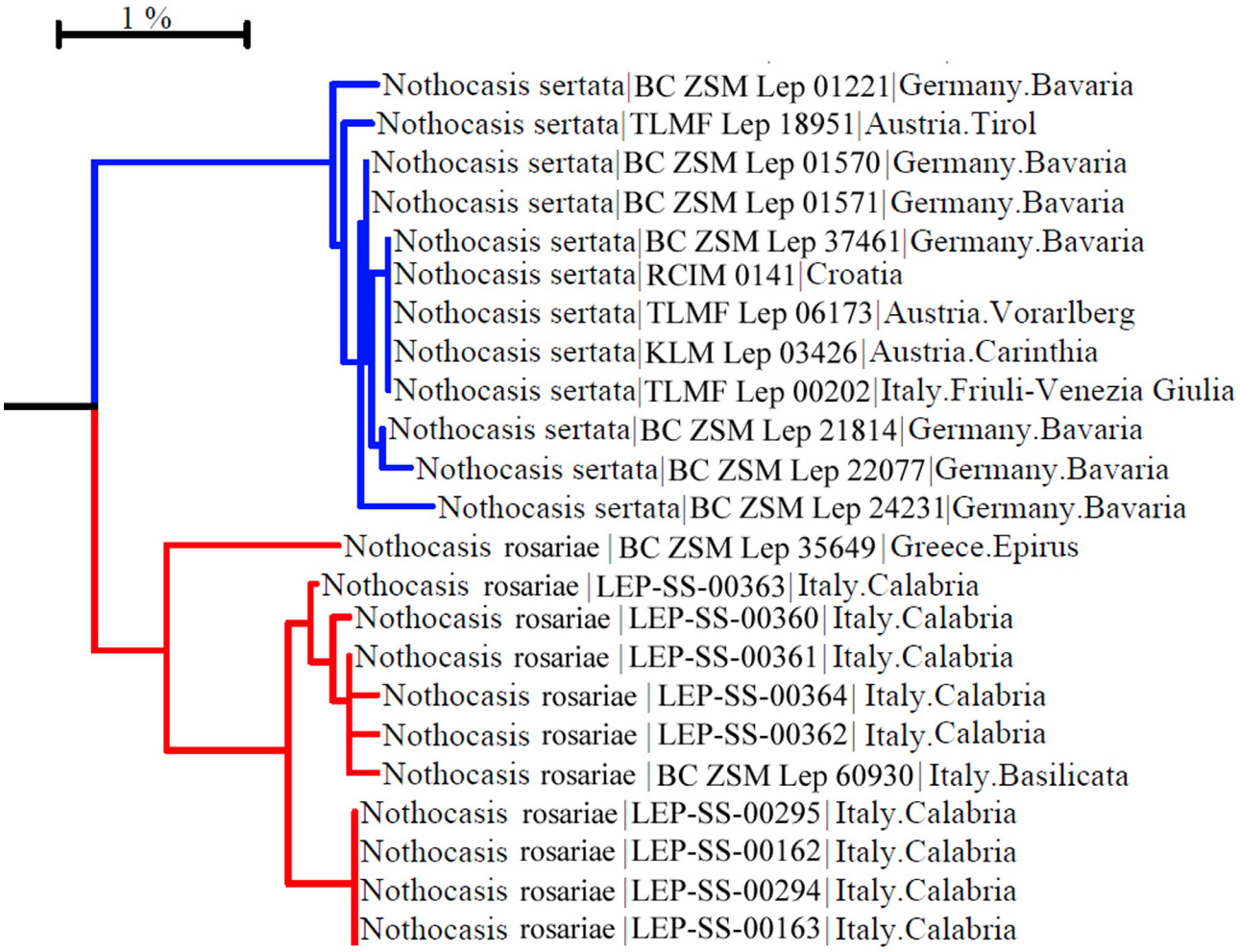


FIGURE 17. Neighbour joining tree (Kimura 2-parameter distance model for COI-5P marker) for 23 European *Nothocasis* specimens. Terminals with specimen ID-number and geography from BOLD

In southern Italy, *Nothocasis sertata* was rarely cited, being recorded for only two localities, one in Campania and one in Basilicata (Parenzan & Hausmann 1992). Recently, in southernmost Basilicata one single specimen could be attributed to *N. rosariae* **sp. n.** through DNA barcoding, but little is known about the Apennine populations north of Basilicata, leaving open the question about the exact position of the boundary between these species.

Despite a comparatively large genetic variation between Italian and Greek populations, the morphology of adults and male genitalia of specimens collected in these countries perfectly matched each other confirming their conspecific status. The largest intra-populational genetic diversity was found in specimens from the Pollino Massif, where we found five different haplotypes (n=5) in a radius of 2.5km. In contrast to that finding, a great genetic homogeneity was observed in the Sila Massif where only one haplotype was found (n=4). This pattern could be the consequence of a recent colonization of the Sila Massif by this species leading to the observed homogeneity (bottleneck/founder effect).

TABLE 1. References of analyzed sequences of *Nothocasis rosariae* sp. n. and *N. sertata* available in BOLD and GenBank.

Species	Country	Region	Area	Specimen ID	Sequence page	GenBank
Nothocasis rosariae	Italy	Calabria	Sila Massif	LEP-SS-00162	BIBSA542-15	KU497383
Nothocasis rosariae	Italy	Calabria	Sila Massif	LEP-SS-00163	BIBSA543-15	KU497388
Nothocasis rosariae	Italy	Calabria	Sila Massif	LEP-SS-00294	BIBSA784-15	KU497384
Nothocasis rosariae	Italy	Calabria	Sila Massif	LEP-SS-00295	BIBSA785-15	KU497381
Nothocasis rosariae	Italy	Calabria	Pollino Massif	LEP-SS-00360	BIBSA850-15	KU497385
Nothocasis rosariae	Italy	Calabria	Pollino Massif	LEP-SS-00361	BIBSA851-15	KU497386
Nothocasis rosariae	Italy	Calabria	Pollino Massif	LEP-SS-00362	BIBSA852-15	KU497387
Nothocasis rosariae	Italy	Calabria	Pollino Massif	LEP-SS-00363	BIBSA853-15	KU497380
Nothocasis rosariae	Italy	Calabria	Pollino Massif	LEP-SS-00364	BIBSA854-15	KU497382
Nothocasis rosariae	Italy	Basilicata	Sirino Massif	BC ZSM Lep 60930	GWOTF364-12	-
Nothocasis rosariae	Greece	Epirus	N Konitza	BC ZSM Lep 35649	GWOSA638-10	-
Nothocasis sertata	Germany	Bavaria	Fuerstenfeldbruck	BC ZSM Lep 01221	GWORB845-07	HQ601437
Nothocasis sertata	Germany	Bavaria	Traunstein	BC ZSM Lep 01570	GWORC1570-08	HQ601436
Nothocasis sertata	Germany	Bavaria	Traunstein	BC ZSM Lep 01571	GWORC1571-08	HQ601438
Nothocasis sertata	Germany	Bavaria	Dietramszell	BC ZSM Lep 21814	GWORK484-09	GU655902
Nothocasis sertata	Germany	Bavaria	Dietramszell	BC ZSM Lep 22077	GWORL365-09	GU686917
Nothocasis sertata	Italy	Friuli-Venezia Giulia	Udine	TLMF Lep 00202	PHLAA162-09	GU689141
Nothocasis sertata	Germany	Bavaria	Berchtesgadener Land	BC ZSM Lep 24231	GWORM182-09	HM903324
Nothocasis sertata	Germany	Bavaria	Regen	BC ZSM Lep 37461	FBLMW360-10	HQ563591
Nothocasis sertata	Croatia	Licko-senjska, Lika	Mt. Licka Pljesivica L2	RCIM 0141	GWOSI141-10	-
Nothocasis sertata	Austria	Vorarlberg	Sonntag, Buchboden	TLMF Lep 06173	PHLSA718-11	KP253253
Nothocasis sertata	Austria	Eastern Tyrol	Lavant, Kienbichl	TLMF Lep 18951	LEATJ1091-15	-
Nothocasis sertata	Austria	Carinthia	Motschula	KLM Lep 03426	ABOLB766-15	-

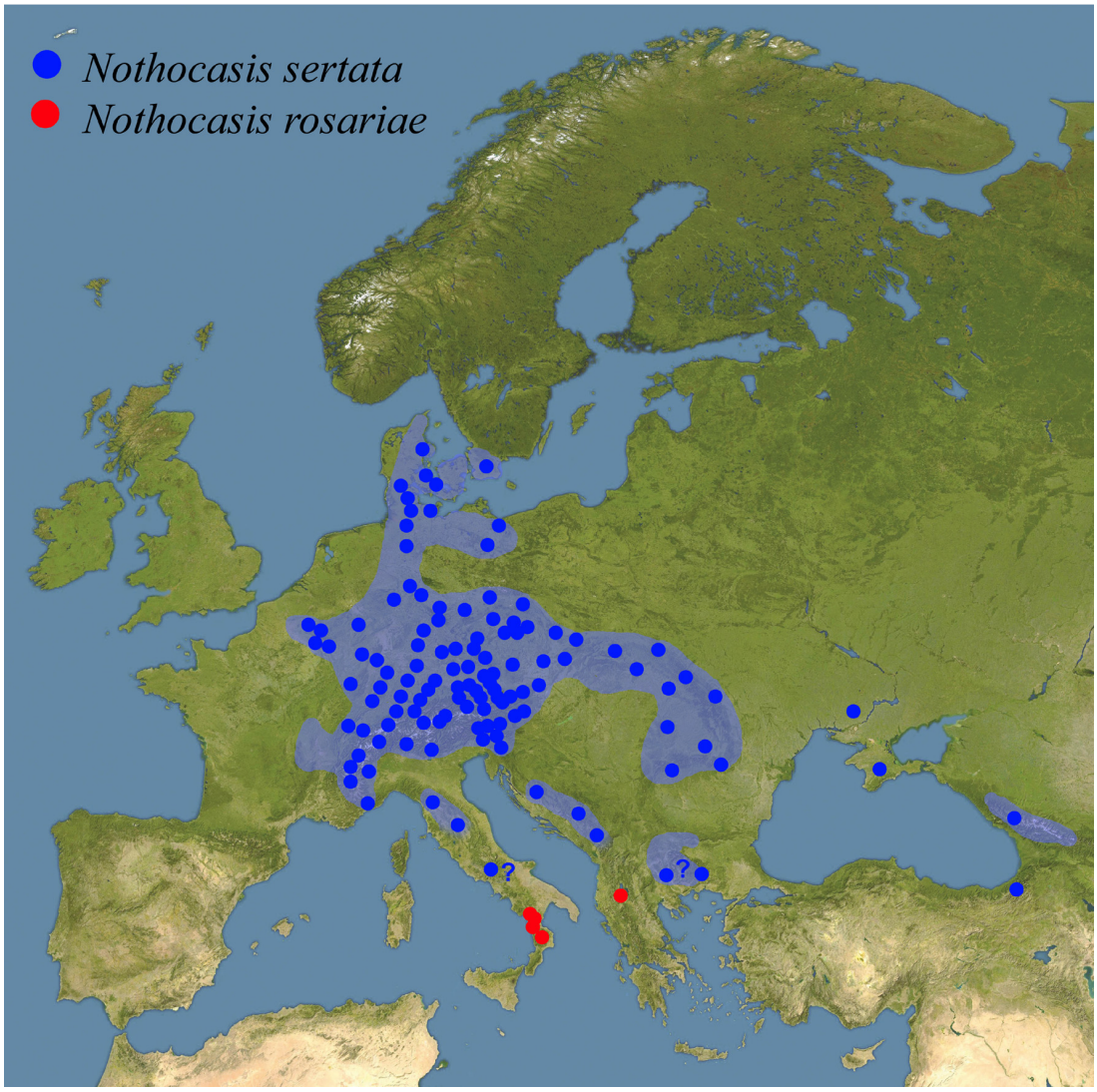


FIGURE 18. Distribution areas of *Nothocasis sertata* (Hübner) and *Nothocasis rosariae* **sp. n.** (from Hausmann & Viidalepp 2012, modified).

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FIGURES 19–20. Habitat of *Nothocasis rosariae* **sp. n.**. **19** Vivaio Sbanditi, *locus typicus*, 1350m, mixed forest, Longobucco, Sila National Park, Italy. **20** Timpone Magara, 1460m, beech forest, Saracena, Pollino National Park, Italy.

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