

***Polietes lardarius* (Fabricius, 1781) & *Polietes meridionalis* Peris & Llorente, 1963 in Belgium (Diptera : Muscidae)**

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Abstract

Polietes meridionalis Peris & Llorente, 1963 (Diptera: Muscidae) is added to the Belgian checklist. This species is very similar to *P. lardarius* (Fabricius, 1781). In this paper we give information about the historical occurrence, the distribution, the flight periods and some ecological aspects of both *Polietes*-species. *Polietes meridionalis* was initially described as a southern, largely Mediterranean vicariant of *P. lardarius*. Given the strong morphological similarity of both species, the question arose as to whether *P. meridionalis* and *P. lardarius* are two distinct species or whether we have to do with a north-south cline of one species. Therefore we re-evaluated the morphological characteristics and analysed the information from mitochondrial COI sequence data.

Keywords : Species new for Belgium, distribution, ecological information, flight periods, DNA-barcoding, Muscidae

Samenvatting

Polietes meridionalis Peris & Llorente, 1963 (Diptera: Muscidae) wordt toegevoegd aan de Belgische checklist. Deze soort lijkt zeer sterk op *P. lardarius* (Fabricius, 1781). In deze publicatie geven we informatie over het historisch voorkomen, de verspreiding, de vliegtijden en een aantal ecologische aspecten van beide *Polietes*-soorten. *Polietes meridionalis* werd initieel beschreven als een zuidelijke, grotendeels Mediterrane vicariant van *P. lardarius*. Gezien de grote morfologische gelijkenis tussen beide soorten, stelt de vraag zich of *P. meridionalis* en *P. lardarius* twee goede soorten zijn of dat er een noord-zuid variatie binnen 1 soort optreedt. Daarom herbekeken we de morfologische kenmerken en analyseerden we de informatie van mitochondrische COI sequentie data.

Résumé

Polietes meridionalis Peris & Llorente, 1963 est ajouté à la liste des Muscidae de Belgique. Cette espèce est très semblable à *P. lardarius* (Fabricius, 1781). Dans cette publication, nous donnons des informations sur l'historique de leur présence, sur leur distribution, les périodes de vol et sur quelques aspects de l'écologie de ces deux espèces de *Polietes*. *Polietes meridionalis* fut initialement décrit comme une espèce du sud, largement méditerranéenne et vicariante de *P. lardarius*. Compte tenu de la forte similarité morphologique des deux espèces, la question s'est posée de savoir si *P. meridionalis* et *P. lardarius* sont deux espèces distinctes ou si nous avons à faire à une variation nord-sud d'une seule espèce. C'est pourquoi nous avons réévalué les caractéristiques morphologiques et analysé l'information à partir de données du séquençage mitochondrial COI.

Introduction

The genus *Polietes* Rondani, 1866 includes medium sized to quite large Muscidae belonging to the tribe Muscini. *Polietes*-species have a M1 vein that is not upcurved as in the other genera of Muscini and the thoracic calypter is narrow (GREGOR *et al.*, 2002). Their larvae are coprophagous and facultatively predaceous in the 2nd and 3rd instar. They prefer droppings of large herbivores (SKIDMORE, 1985; GREGOR *et al.*, 2002).

The Belgian Muscidae catalogue (HOFMANS, 1991) contains three *Polietes*-species: *P. domitor* (Harris, 1780), *P. lardarius* (Fabricius, 1781) and *P. steinii* (Ringdahl, 1913). In this paper we add *Polietes meridionalis* Peris & Llorente, 1963, a species that is very similar to *P. lardarius*.

Polietes meridionalis was initially described as a southern, largely Mediterranean species, while *P. lardarius* occurs in Central and Northern Europe. Subsequently *P. meridionalis* has been found to be widespread in the Mediterranean subregion and to extend also into the warmer parts of lowland Central and Western Europe (PERIS & LLORENTE, 1963; PONT, 2013). Nowadays the species is known in Europe from Albania, Britain, Bulgaria, Crete, Croatia, Cyprus, the French mainland, Germany, the Greek mainland, Hungary, the Italian mainland, the Netherlands, the Portuguese mainland, Sicily, the Spanish mainland and Ukraine. Outside of Europe the species occurs in the Near East and in North Africa (PONT, 2013; PONT & FALK, 2013).

Given their strong morphological similarity the question arose as to whether *P. meridionalis* and *P. lardarius* are two good species or whether we have to do with a north-south cline of one species. Therefore we made a synthesis of the morphological characteristics proposed by several authors and we re-evaluated these characteristics while identifying the Belgian material. In addition we assessed the divergence at the mitochondrial COI marker between both species.

In this paper we give also some information about the distribution, the flight period and the habitat preferences of both species.

Observations *Polietes lardarius* and *Polietes meridionalis* in Belgium

Polietes meridionalis was recognised for the first time in Belgium in 2011 by the first author. In 2011 and 2012 she collected specimens and noted systematically all field observations of both *Polietes meridionalis* and *P. lardarius*. Subsequently the Muscidae collections of Charel Verbeke, Joris Menten and the Royal Belgian Institute of Natural Sciences (RBINS) were assessed. After a call for extra observations of *P. meridionalis*, a few additional records from other collectors could be added (only specimens of which we were able to confirm the identification were considered here). Below we give the details of all records. All collected specimens are dry mounted, unless stated otherwise. An asterisk indicates that the specimen was used for DNA-analysis.

Belgian Records

Polietes lardarius

WEST FLANDERS: Jabbeke, Snellegem, 25.VII.1976, Collected: 1♂, Leg. F. Verbeke, Coll. C. Verbeke, Det. C. Martens; Jabbeke, Snellegem, XI.1976, Collected: 3♀, Leg. & Coll. C. Verbeke, Det. C. Martens; Brugge, 25.V.1978, Collected: 2♀, Leg. & Coll. C. Verbeke, Det. C. Martens; Zedelgem, 28.V.1978, Collected: 1♀, Leg. & Coll. C. Verbeke, Det. C. Martens; Zedelgem, Loppem, 14.VI.1978, Collected: 1♀, Leg. & Coll. C. Verbeke, Det. C. Martens; Brugge, Homestead, 23.X.1978, Collected: 1♀, Leg. & Coll. C. Verbeke, Det. C. Martens; Beernem, Bulskampveld, 15.IX.2011, Field observation: 1♀ sunbasking on a leaf, Collected: 1♀, Leg., Coll. & Det. C. Martens; Beernem, Bulskampveld - Herb Garden, 29.X.2011, Field observation: 1♀, Collected: 1♀, Leg., Coll. & Det. C. Martens; Damme, Rijckevelde Meersbeek Noord, 19.X.2012, Field observation: 1♂ on *Hedera helix* L., Collected: 1*♂ on alcohol, Leg. C. Martens, Coll. RBINS, Det. C. Martens; Wingene, Vagevuurbossen - Heideveld Boskapeldreef, 18.XI.2012, Field observation: 5+ sunbasking on trunk of *Fagus sylvatica* L., Leg., Coll. & Det. C. Martens.

EAST FLANDERS: Eeklo, 28.V.1911, Collected: 1♂, Leg. M. Goetghebuer, Coll. RBINS, Det. C. Martens; Gent, 9.V.1912, Collected: 1♀, Leg. M. Goetghebuer, Coll. RBINS, Det. C. Martens; Waarschoot, 9.VII.1922, Collected: 1♀, Leg. M. Goetghebuer, Coll. RBINS, Det. C. Martens; Merelbeke, Schelderode, 10.V.1941, Collected: 1♂, Leg. J. Verbeke, Coll. C. Verbeke, Det. C. Martens; Waarschoot, 26.V.1943, Collected: 1♀, Leg. J. Verbeke, Coll. C. Verbeke, Det. C. Martens; Melle, 7.VI.1943, Collected: 1♂, Leg. J. Verbeke, Coll.

C. Verbeke, Det. C. Martens; Destelbergen, Heusden, 17.VII.1943, Collected: 1♀, Coll. C. Verbeke, Det. C. Martens; Destelbergen, 8.VIII.1943, Collected: 1♂, Coll. C. Verbeke, Det. C. Martens; Merelbeke, Schelderode, 29.V.1944, Collected: 1♀, Leg. J. Verbeke, Coll. C. Verbeke, Det. C. Martens; Melle, 11.VI.1944, Collected: 1♀, Leg. J. Verbeke, Coll. C. Verbeke, Det. C. Martens; Aalter, Bellem, 16.VIII.1944, Collected: 1♂, Leg. J. Verbeke, Coll. C. Verbeke, Det. C. Martens; Destelbergen, 27.V.1946, Collected: 1♀, Leg. J. Verbeke, Coll. C. Verbeke, Det. C. Martens; Melle, 27.X.1948, Collected: 1♀, Leg. M. Goetghebuer, Coll. RBINS, Det. C. Martens; Maldegem, Burkel Zuid, 18.V.2011, Field observation: 1♀, Collected: 1♀, Leg., Coll. & Det. C. Martens; Knesselare, Garden Koffiestraat 6, 10.XI.2011, Field observation: 2♀ on *Hedera helix*, Collected: 2♀, Leg., Coll. & Det. C. Martens; Maldegem, Maldegemveld Zuid, 7.VI.2012, Field observation: 1, Det. C. Martens; Knesselare, Drongengoed Midden, 11.XI.2012, Field observation: 10+, Det. C. Martens.

ANTWERP: Schilde, 's Gravenwezel, 7.IX.1942, Collected: 1♀, Leg. J. Verbeke, Coll. C. Verbeke, Det. C. Martens; Zoersel, Sint-Antonius, 30.V.1943, Collected: 1♂, Coll. C. Verbeke, Det. C. Martens; Antwerpen, Hoboken, 1.X.1949, Collected: 1♀, Leg. L. Marnef, Coll. RBINS, Det. C. Martens.

FLEMISH BRABANT: Sint-Pieters-Leeuw, 10.V.1874, Collected: 1♂, Leg. J. Jacobs, Coll. RBINS, Det. C. Martens; Beersel, 8.VI.1933, Collected: 1♂, Leg. A. Collart, Coll. RBINS, Det. C. Martens; Oud-Heverlee, Oud-Heverlee Zuid, 20.V.2002, Collected: 1♂, Leg., Coll. & Det. J. Menten; Hoeilaart, Sonian Forest, Near Ponds Duboislaan Groenendaal, 25.V.2012, Field observation: 1 flying trough herbaceous vegetation, Det. C. Martens; Bierbeek, Meerdaalwoud - Pruikenmakers & Dikke Eikdreef, 27.V.2012, Field observation: 10+ on saprun, Collected: 3♀ and 4♂ on alcohol, Leg. C. Martens, Coll. RBINS (1*♂) & C. Martens (rest), Det. C. Martens; Oud-Heverlee, Meerdaalwoud - Warande, 27.V.2012, Field observation: 10+ on droppings of horses, Det. C. Martens; Oudergem, Sonian Forest - Rood Klooster, 14.VI.2012, Field observation: 2, Collected: 1*♂ on alcohol, Leg. C. Martens, Coll. RBINS, Det. C. Martens.

HAINAUT: Mons, V.1900, Collected: 2♂, Leg. A. Guillaume, Coll. RBINS, Det. C. Martens; Flobecq, 24.VIII.1942, Collected: 1♂, Coll. C. Verbeke, Det. C. Martens; Braine-le-Comte, La Houssière, Collected: 1♀, Leg. P. de Moffarts, Coll. RBINS, Det. C. Martens.

NAMUR: Profondeville, Lustin, 27.IX.1946, Collected: 1♂ and 21♀ on *Phallus impudicus* L.:Pers., Leg. A. Collart, Coll. RBINS, Det. C. Martens; Viroinval, Tienne aux Boulis, 27.XI.2011-8.I.2012, Collected: 1♀ from pitfall trap, Leg. R. Kekenbosch, Coll. C. Martens, Det. C. Martens; Andenne, Réserve Naturelle de Sclaigneau, 2.VI.2012, Field observation: 5, Collected: 1♂ dry and 1*♂ on alcohol, Leg. C. Martens, Coll. RBINS (specimen on alcohol) & C. Martens (dry specimen), Det. C. Martens.

LIÈGE: Hertogenwald, 29/9/1891, Collected: 1♀, Leg. E. Candèze, Coll. RBINS, Det. C. Martens; Chaudfontaine, Embourg, 15.IX.1895, Collected: 2♀, Leg. E. Candèze, Coll. RBINS, Det. C. Martens; Esneux, Tilff, 29.VI.1926, Collected: 1♂, Coll. RBINS, Det. C. Martens; Plombières, Moresnet, 10.IX.1934, Collected: 1♀, Leg. A. Collart, Coll. RBINS, Det. C. Martens; Malmédy, Bois du Calvaire, 25.VI.1935, Collected: 1♂ and 1♀, Leg. A. Collart, Coll. RBINS, Det. C. Martens; Herve, Val Dieu, 3.VI.1938, Collected: 1♂, Leg. J. Muller, Coll. RBINS, Det. C. Martens; Visé, 9.VII.1938, Collected: 1♀, Leg. J. Muller, Coll. RBINS, Det. C. Martens; Dalhem, Warsage, 11.X.1938, Collected: 1♀, Leg. J. Muller, Coll. RBINS, Det. C. Martens; Visé, Jardin, 6.X.1939, Collected: 1♀, Leg. J. Muller, Coll. RBINS, Det. C. Martens; Beyne-Heusay, Beyne, 21.V.1942, Collected: 1♂, Coll. RBINS, Det. C. Martens; Ferrières, Vieuxville, Sy, 24.V.1946, Collected: 14♂ and 5♀, Leg. A. Collart, Coll. RBINS, Det. C. Martens; Beyne-Heusay, 25.V.1953, Collected: 1♂, Coll. RBINS, Det. C. Martens; Braives, Fallais, 23.VI.1979, Collected: 1♀, Leg. H. Donckier, Coll. RBINS, Det. C. Martens; Waimes, Plateau des Hautes-Fagnes - Intersection Route de Botrange & Rue d'Averscheidt, 18.VI.2012, Field observation: 25+, Collected: 5♂ dry and 2♂ on alcohol, Leg. C. Martens, Coll. RBINS (1*♂ on alcohol) & C. Martens (rest), Det. C. Martens; Büllingen, Vallée de la Holzwarche at Rocherath, 22.VI.2012, Field observation: 1, Det. C. Martens; Büllingen, Vallée de la Holzwarche at Rocherath, 23.VI.2012, Field observation: 100+, Collected: 4♂ on alcohol, Leg. C. Martens, Coll. RBINS (1*) & C. Martens (rest), Det. C. Martens; Baelen, Drossart, 18.VII.2012, Field observation: 1 flying trough herbaceous vegetation, Det. C. Martens; Bütgenbach, Rurbusch, 18.VII.2012, Field observation: 5+, Det. C. Martens.

LUXEMBOURG: Manhay, 4.IV.1894, Collected: 1♂, Leg. E. Candèze, Coll. RBINS, Det. C. Martens; Vielsalm, 29.VIII.1895, Collected: 1♂, Leg. E. Candèze, Coll. RBINS, Det. C. Martens; Virton, 20.VI.1909, Collected: 1♂, Leg. M. Goetghebuer, Coll. RBINS, Det. C. Martens; Virton, 18.VI.1910, Collected: 2♀, Leg. M. Goetghebuer, Coll. RBINS, Det. C. Martens; Chiny, 25.VI-8.VII.1945, Collected: 1♂, Leg. R. Laurent, Coll. RBINS, Det. C. Martens; Rouvroy, Torgny, 20.V.1952, Collected: 1♀, Coll. C. Verbeke, Det. C. Martens.

Polietes meridionalis

WEST FLANDERS: Jabbeke, Snellegem, 25.VII.1976, Collected: 1♂, Leg. F. Verbeke, Coll. C. Verbeke, Det. C. Martens; Jabbeke, Snellegem, X.1976, Collected: 1♀, Leg. F. Verbeke, Coll. C. Verbeke, Det. C. Martens; Brugge, 25.V.1978, Collected: 1♂, Leg. & Coll. C. Verbeke, Det. C. Martens; Zedelgem, 27.V.1978, Collected: 1♀, Leg. & Coll. C. Verbeke, Det. C. Martens; Jabbeke, Snellegem, 4.VI.1978, Collected: 2♀, Leg. & Coll.

C. Verbeke, Det. C. Martens; Brugge, 20.VI.1978, Collected: 2♂, Leg. & Coll. C. Verbeke, Det. C. Martens; Brugge, 30.VI.1978, Collected: 1♂, Leg. & Coll. C. Verbeke, Det. C. Martens; Zedelgem, 12.VII.1978, Collected: 2♂, Leg. & Coll. C. Verbeke, Det. C. Martens; Jabbeke, Snellegem, Homestead, 18.IX.1980, Collected: 1♂ and 1♀, Leg. & Coll. C. Verbeke, Det. C. Martens; Jabbeke, Snellegem, 23.X.1981, Collected: 1♀, Leg. & Coll. C. Verbeke, Det. C. Martens; De Haan, 15.XI.1981, Collected: 1♀, Leg. & Coll. C. Verbeke, Det. C. Martens; Oudenburg, Westkerke, 27.VIII.1983, Collected: 1♂, Leg. G. Haghebaert, Coll. C. Verbeke, Det. C. Martens; Brugge, Crematorium Blauwe Toren, 11.VI.2011, Field observation: 1♀, Collected: 1♀, Leg., Coll. & Det. C. Martens; Ruiselede, Disveld, 19.VI.2011, Field observation: 1♀, Collected: 1♀, Leg., Coll. & Det. C. Martens; Beernem, Bulskampveld - Herb Garden, 15.IX.2011, Field observation: 4♂ and 2♀ on *Sedum telephium* L. and *Foeniculum vulgare* Mill., Collected: 4♂ and 2♀, Leg., Coll. & Det. C. Martens; Beernem, Miseriebocht, 18.IX.2011, Field observation: 1♂ on *Senecio jacobaea* L., Collected: 1♂, Leg., Coll. & Det. C. Martens; De Panne, Centre of Adinkerke, 27.IX.2011, Field observation: 1♂ on *Hedera helix*, Collected: 1♂, Leg., Coll. & Det. J. Mortelmans; Beernem, Bulskampveld - Herb Garden, 29.X.2011, Field observation: 155, Collected: 1♀, Leg., Coll. & Det. C. Martens; Beernem, Rijckvelde West, 30.XI.2011, Field observation: 19, Collected: 7♀, Leg., Coll. & Det. C. Martens; Brugge, Schobbejakshoogte West, 30.XI.2011, Field observation: 19, Collected: 2♀, Leg., Coll. & Det. C. Martens; Beernem, Rijckvelde West, 9.XII.2011, Field observation: 52 sunbasking on trunks of a wide variety of tree species, Leg., Coll. & Det. C. Martens; Brugge, Schobbejakshoogte West, 9.XII.2011, Field observation: 8 sunbasking on trunks of *Quercus robur* L., Det. C. Martens; Brugge, Schobbejakshoogte West, 9.XII.2011, Field observation: 8 sunbasking on trunks of a variety of tree species, Det. C. Martens; Beernem, Bulskampveld - Near hoeve Colpaert, 7.X.2012, Field observation: 25+, Collected: 3♂ on alcohol, Leg. C. Martens, Coll. RBINS (1*) & C. Martens (rest), Det. C. Martens; Damme, Rijckvelde Meersbeek Noord, 19.X.2012, Field observation: 2 on *Hedera helix*, Det. C. Martens; Beernem, Miseriebocht, 28.X.2012, Field observation: 1, Det. C. Martens; Wingene, Vagevuurbossen - Heideveld Boskapeldreef, 18.XI.2012, Field observation: 3 on *Heracleum sphondylium* L. and sunbasking on trunk of *Fagus sylvatica*, Det. C. Martens;

EAST FLANDERS: Aalst, Moorsel, 1.X.1933, Collected: 1♂, Leg. A. Ball, Coll. RBINS, Det. C. Martens; Aalst, Moorsel, 4.X.1933, Collected: 2♂, Leg. A. Ball, Coll. RBINS, Det. C. Martens; Maldegem, Drongengoed Noord, 28.V.2011, Field observation: 1♀, Collected: 1♀, Leg., Coll. & Det. C. Martens; Maldegem, Maldegemveld Zuid, 3.VI.2011, Field observation: 1♀, Collected: 1♀, Leg., Coll. & Det. C. Martens; Maldegem, Maldegemveld Zuid, 4.VI.2011, Field observation: 1♀, Collected: 1♀, Leg., Coll. & Det. C. Martens; Maldegem, Maldegemveld Noord, 4.VI.2011, Field observation: 1♀, Collected: 1♀, Leg., Coll. & Det. C. Martens; Knesselare, Vliegveld, 10.IX.2011, Field observation: 1, Collected: 1♀, Leg., Coll. & Det. C. Martens; Maldegem, Maldegemveld Zuid, 10.IX.2011, Field observation: 1♂ on *Angelica sylvestris* L., Collected: 1♂, Leg., Coll. & Det. C. Martens; Knesselare, Garden Koffiestraat 6, 10.IX.2011, Field observation: 3♂ and 1♀ on *Hedera helix*, Collected: 3♂ and 1♀, Leg., Coll. & Det. C. Martens; Knesselare, Garden Koffiestraat 6, 21.IX.2011, Field observation: 2♂, Collected: 2♂, Leg., Coll. & Det. C. Martens; Knesselare, Garden Koffiestraat 6, 22.IX.2011, Field observation: 24, Det. C. Martens; Knesselare, Garden Koffiestraat 6, 23.IX.2011, Field observation: 20♂ and 4♀, Collected: 20♂ and 4♀, Leg., Coll. & Det. C. Martens; Eeklo, Garden Lazarijstraat, 23.IX.2011, Field observation: 1♀ on *Hedera helix*, Det. C. Bruggeman & C. Martens; Knesselare, Drongengoed Midden, 25.IX.2011, Field observation: 1 sunbasking on the ground, Det. C. Martens; Knesselare, Drongengoed Midden, 25.IX.2011, Field observation: 1 sunbasking on the ground, Det. C. Martens; Knesselare, Garden Koffiestraat 6, 27.IX.2011, Field observation: 29 on *Hedera helix*, Det. C. Martens; Knesselare, Garden Koffiestraat 6, 27.IX.2011, Field observation: 10, Det. C. Martens; Waarschoot, Het Leen at Waarschoot, 29.IX.2011, Field observation: 3 flying around and sunbasking on the ground, Det. C. Martens; Knesselare, Garden Koffiestraat 6, 30.IX.2011, Field observation: 3, Det. C. Martens; Maldegem, Maldegemveld Zuid, 1.X.2011, Field observation: 9, Det. C. Martens; Maldegem, Maldegemveld Zuid, 13.X.2011, Field observation: 100+ on droppings of Galloway cattle, Collected: 6♀, Leg., Coll. & Det. C. Martens; Knesselare, Garden Koffiestraat 6, 15.X.2011, Field observation: 2♂ and 1♀ on *Hedera helix*, Collected: 2♂ and 1♀, Leg., Coll. & Det. C. Martens; Aalter, Taborschool Bellem, 24.X.2011, Field observation: 1♀ on *Hedera helix*, Det. M. Vanmoerkerke & C. Martens; Knesselare, Garden Koffiestraat 6, 28.X.2011, Field observation: 10♀, Collected: 10♀, Leg., Coll. & Det. C. Martens; Knesselare, Garden Koffiestraat 6, 10.XI.2011, Field observation: 10♀ on *Hedera helix*, Collected: 10♀, Leg., Coll. & Det. C. Martens; Knesselare, Garden Koffiestraat 6, 21.XI.2011, Field observation: 32 on *Hedera helix*, Collected: 17♀, Leg., Coll. & Det. C. Martens; Knesselare, Garden Koffiestraat 6, 28.XI.2011, Field observation: 2♀ on *Hedera helix*, Collected: 2♀, Leg., Coll. & Det. C. Martens; Aalter, Small private forest near Ganzeveld, 3.VII.2012, Field observation: 1♂, Collected: 1♂ on alcohol, Leg. C. Martens, Coll. RBINS, Det. C. Martens; Deinze, Garden Ten Bosse, 4.IX.2012, Field observation: 1♂, Collected: 1♂, Leg. & Coll. K. Verhoeyen, Det. K. Verhoeyen & C. Martens; Assenede, Doornendijkstraat, 9.IX.2012, Field observation: 1♂, Collected: 1*♂ on

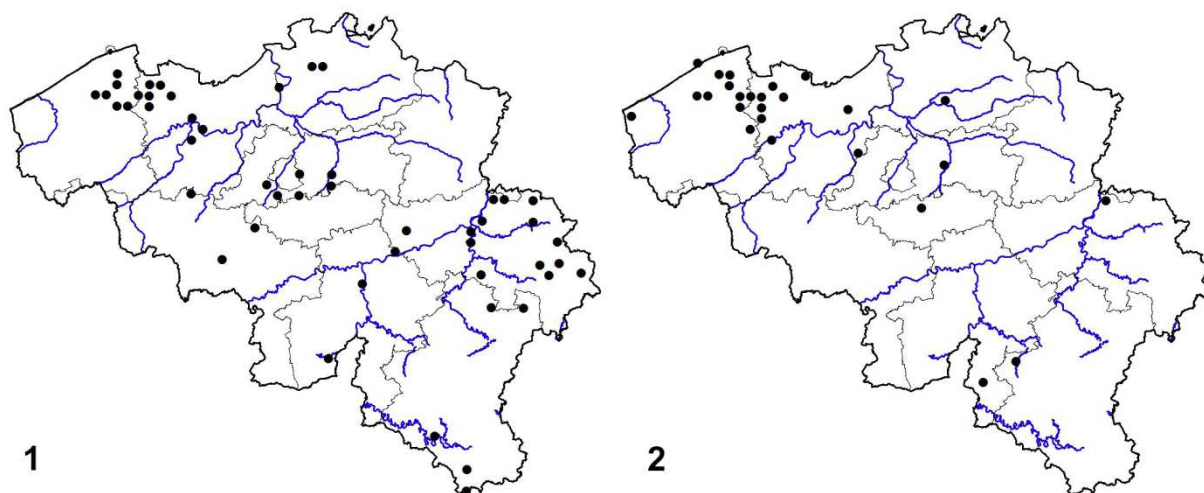


Fig. 1. Distribution map for *Polietes lardarius* (based on 5 km UTM squares).

Fig. 2. Distribution map for *Polietes meridionalis* (based on 5 km UTM squares).

alcohol, Leg. C. Martens, Coll. RBINS, Det. C. Martens; Knesselare, Drongengoed Midden, 19.IX.2012, Field observation: 100+ mainly on *Hedera helix*, Collected: 11♂ on alcohol, Leg. C. Martens, Coll. RBINS (1*) & C. Martens (rest), Det. C. Martens; Knesselare, Garden Koffiestraat 6, 30.IX.2012, Field observation: 5+, Collected: 4♂ on alcohol, Leg. C. Martens, Coll. RBINS (1*) & C. Martens (rest), Det. C. Martens; Lokeren, Molsbroek, 1.X.2012, Field observation: 1♂ on *Hedera helix*, Collected: 1♂, Leg., Coll. & Det. J. Mortelmans; Knesselare, Drongengoed Midden, 11.XI.2012, Field observation: 2, Det. C. Martens.

ANTWERP: Nijlen, Kleine Netevallei - Het Goor, 3.IX.1993, Collected: 1♂, Leg., Coll. & Det. J. Menten.

FLEMISH BRABANT: Bertem, Koeheide, 25.V.2002, Collected: 1♀, Leg., Coll. & Det. J. Menten.

WALLOON BRABANT: Rixensart, VI.1903, Collected: 1♀, Leg. A. Guillaume, Coll. RBINS, Det. C. Martens.

NAMUR: Gedinne, Louette-Saint-Pierre, 15.IV.1970, Collected: 1♂, Leg. Gravet, Coll. RBINS, Det. C. Martens.

LIÈGE: Visé, 17.X.1939, Collected: 1♀, Leg. J. Muller, Coll. RBINS, Det. C. Martens; Visé, 21.X.1939, Collected: 1♀, Leg. J. Muller, Coll. RBINS, Det. C. Martens.

LUXEMBOURG: Wellin, Forest 2 km south of the centre, 1.XI.2011, Field observation: 50 on *Hedera helix*, Collected: 7♂, Leg., Coll. & Det. J. Mortelmans.

Historical occurrence

The first Belgian record of *Polietes lardarius* dates from 1874 and the first Belgian record of *Polietes meridionalis* dates from 1903. Before 1970 *P. meridionalis* was recorded only sporadically. For this time period there are only 5 records and only 6 specimens (3♂ and 3♀) of *P. meridionalis*, while there are 39 records and 82 specimens (35♂ and 47♀) of *P. lardarius*. However, starting from the 1970s *Polietes meridionalis* has been recorded more often than *P. lardarius*. In 2011-2012 the number of *meridionalis* records is even 2.5 times the number of *lardarius* records (47 versus 19) and the average number of *meridionalis* specimens observed in the field per record is 1.2 times that for *lardarius* (18.7 versus 15.9).

Distribution

Figures 1 and 2 give distribution maps for *Polietes lardarius* and *Polietes meridionalis*, based on 5 km UTM squares. The records of both species are obviously scattered over the country. The large number of observations and occupied UTM squares for West- and East-Flanders reflects the residence of the main collectors. The provinces of Limburg, Walloon Brabant and Hainaut are subsampled.

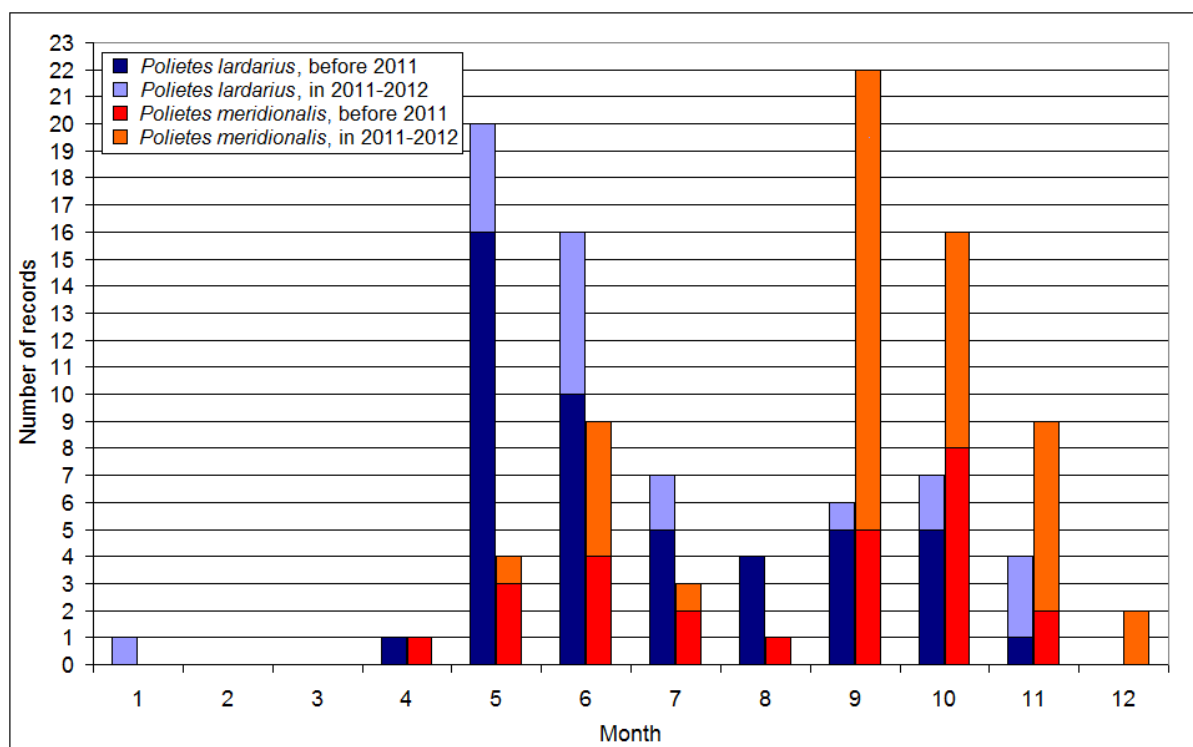


Fig. 3. Evolution throughout the year of the number of records of *Polietes lardarius* and *Polietes meridionalis*.

Habitats

Both species were found in a similar variety of habitats (forests, heath, gardens...) and were even sometimes found together at the same place and time (eg. Herb garden Bulskampveld in Beernem at 29.X.2011, Garden in the centre of Knesselare at 10.XI.2011, Schobbejakshoogte in Brugge at 9.XII.2011, Rijckevelde Meersbeek Noord in Damme at 19.X.2012, Drongengoed Midden in Knesselare at 11.XI.2012 and Vagevuurbossen - Heideveld Boskapeldreef in Wingene at 18.XI.2012). *Polietes lardarius* has been observed on a sap run, on droppings of horses, on *Phallus impudicus* and on *Hedera helix*. *Polietes meridionalis* was mostly feeding on flowers (*Hedera helix*, *Angelica sylvestris*, *Heracleum sphondylium*, *Sedum telephium*, *Foeniculum vulgare* and *Senecio jacobaea*), but has been observed also on droppings of Galloway cattle. In autumn large numbers of especially *P. meridionalis* occur on *Hedera helix* for as long as it flowers. Later in the year they are mainly found basking in the sun on trunks and leaves. Field experience of the first author indicates that at the end of the year most flies are females, but absolute data on the sex ratio are not available.

Flight periods

Figure 3 shows how the number of records evolves throughout the year. Figure 4 shows how the average number of specimens present in the field per record evolves throughout the year. The latter figure is based only on data from 2011-2012, as this information is not available for earlier records. Both species seem to occur from April (first record on 4 April for *P. lardarius* and on 15 April for *P. meridionalis*) until about the end of the year. There is a first peak in spring (May-June for *P. lardarius* and June for *P. meridionalis*) and a second one in autumn (September-November). For *P. lardarius* the first peak seems to be most important. But *P. meridionalis* is recorded more frequently and in higher numbers in autumn.

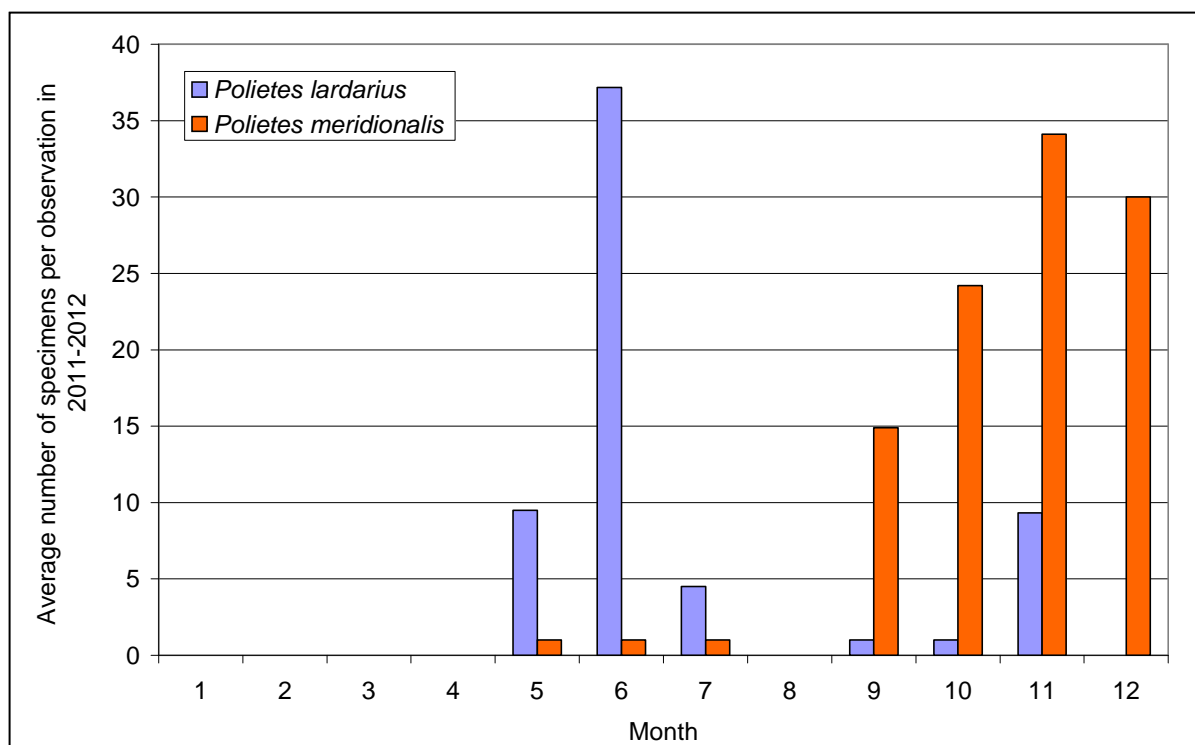


Fig. 4. Evolution throughout the year of the average number of specimens of *Polietes lardarius* and *Polietes meridionalis* per observation in 2011-2012.

Distinctions between *Polietes lardarius* and *P. meridionalis*

Morphological characteristics

The morphological characteristics of both species, as given by several authors, are listed in table 1. We verified these characteristics while identifying the Belgian material and added some remarks.

The colour of the anterior spiracle and the colour of the anterior part of the head are reliable characteristics, which, with some experience, are also useful for identification in the field. The definition of the colours differs slightly from author to author, but in any case the anterior spiracle is yellow and sometimes partly yellowish-white in *P. meridionalis*, while it is brown and often partly greyish (according to some authors it can be also greyish-black or brownish-black) in *P. lardarius*. The colour of the anterior part of the head is golden yellow (according to some authors it can be also yellowish-white) in *P. meridionalis* and partly brown, partly greyish-white (according to some authors it can be also silvery white) in *P. lardarius*. The shape of the lower calypter and the shape of the cercal plate are good characteristics for the identification of males under the microscope, but are not useful as field characteristics. The width of the parafacials gives a good indication, but is subject to some variation. In our experience the colour of the setae on the postgena is not always reliable and the number of anteroventral setae on the hind tibia is too variable to be really useful.

Morphological differentiation

- Anterior spiracle brown, often partly greyish. Anterior part of head partly brown, partly greyish-white. In male hind margin of lower calypter diverging from margin of scutellum at angle of ca. 90°, when fly set with wings extended at right angles from the body. Secondary characters (subject to some variation): width of parafacial at level of insertion of arista in male ca. 1.0 times and in female ca. 1.25 times width of postpedicel, setae on postgena mostly all black (but sometimes with some brown setae), in male (3-)4-7(-8) and in female (2-)3-5(-6) anteroventral setae on hind tibia.

P. lardarius

- Anterior spiracle yellow, sometimes partly yellowish-white. Anterior part of head golden yellow. In male hind margin of lower calypter diverging from margin of scutellum at angle of much less

than 90°, when fly set with wings extended at right angles from the body. Secondary characters (subject to some variation): width of parafacial at level of insertion of arista in male ca. 1.2 times and in female ca. 1.5-1.75 times width of postpedicel, postgena mostly with some pale (whitish or brownish) setae, in male 2-6 and in female 2-4 anteroventral setae on hind tibia.

P. meridionalis

Genetic differences

LAB PROTOCOLS

DNA barcodes based on the mitochondrial cytochrome c oxidase subunit I gene (COI) have been shown to be a highly efficient tool for identification of northern Nearctic muscid flies (RENAUD *et al.*, 2012). Here, it is assumed to be applicable for Palearctic species too. In total, eight specimens of *Polietes lardarius* and nine specimens of *Polietes meridionalis* were caught for DNA analysis and

Table 1. Morphological characteristics of *Polietes lardarius* and *Polietes meridionalis*.

		PERIS & LLORENTE, 1963	GREGOR <i>et al.</i> , 2002	PONT & FALK, 2013	remarks based on study of Belgian material
♂ & ♀	anterior spiracle	<i>lardarius</i> : grey or greyish-black <i>meridionalis</i> : yellowish-white or yellow	<i>lardarius</i> : greyish-brown to brownish-black <i>meridionalis</i> : yellowish	<i>lardarius</i> : grey or greyish-black <i>meridionalis</i> : yellowish-white or white	<i>lardarius</i> : brown, often partly greyish <i>meridionalis</i> : yellow, sometimes partly yellowish-white
	anterior part of head	<i>lardarius</i> : grey or silvery-white <i>meridionalis</i> : yellowish white or golden yellow	<i>lardarius</i> : whitish to grey <i>meridionalis</i> : yellowish white to golden yellow	<i>lardarius</i> : grey or silvery-white, rarely with some brown <i>meridionalis</i> : whitish-yellow or golden	<i>lardarius</i> : partly brown, partly greyish-white <i>meridionalis</i> : golden yellow
	setae on postgena			<i>lardarius</i> : all setae black <i>meridionalis</i> : some long pale setae	<i>lardarius</i> : mostly all setae black, but sometimes with some brown setae <i>meridionalis</i> : number of pale setae very variable and in exceptional cases pale setae even absent; pale setae mostly whitish, but sometimes brownish
♂	anteroventral setae on hind tibia	<i>lardarius</i> : 6-7 <i>meridionalis</i> : usually 4	<i>lardarius</i> : usually 6-7 <i>meridionalis</i> : usually 4	very variable <i>lardarius</i> : 6-7 <i>meridionalis</i> : usually 4-5	<i>lardarius</i> : (3-)-4-7(-8) <i>meridionalis</i> : 2-6
	width of parafacial (at level of insertion of arista, when seen in lateral view)	<i>lardarius</i> : slightly narrower than postpedicel <i>meridionalis</i> : slightly broader than postpedicel	<i>lardarius</i> : narrower than postpedicel <i>meridionalis</i> : broader than postpedicel	<i>lardarius</i> : 1.0 times width of postpedicel <i>meridionalis</i> : 1.2 times width of postpedicel	
	lower calypter (fly set with wings extended at right angles from the body)			<i>lardarius</i> : hind margin diverging from margin of scutellum at angle of ca. 90° <i>meridionalis</i> : hind margin diverging from margin of scutellum at angle of much less than 90°	
	shape of cercal plate	<i>lardarius</i> : see Fig. 10 in PERIS & LLORENTE (1963) <i>meridionalis</i> : see Fig. 9 in PERIS & LLORENTE (1963)		<i>lardarius</i> : each half of the plate has a weakly concave apical margin <i>meridionalis</i> : apex is more sloping and rounded than in <i>lardarius</i>	
♀	anteroventral setae on hind tibia			very variable	<i>lardarius</i> : (2-)-3-5(-6) <i>meridionalis</i> : 2-4
	width of parafacial (at level of insertion of arista, when seen in lateral view)			<i>lardarius</i> : 1.25 times width of postpedicel <i>meridionalis</i> : 1.5-1.75 times width of postpedicel	

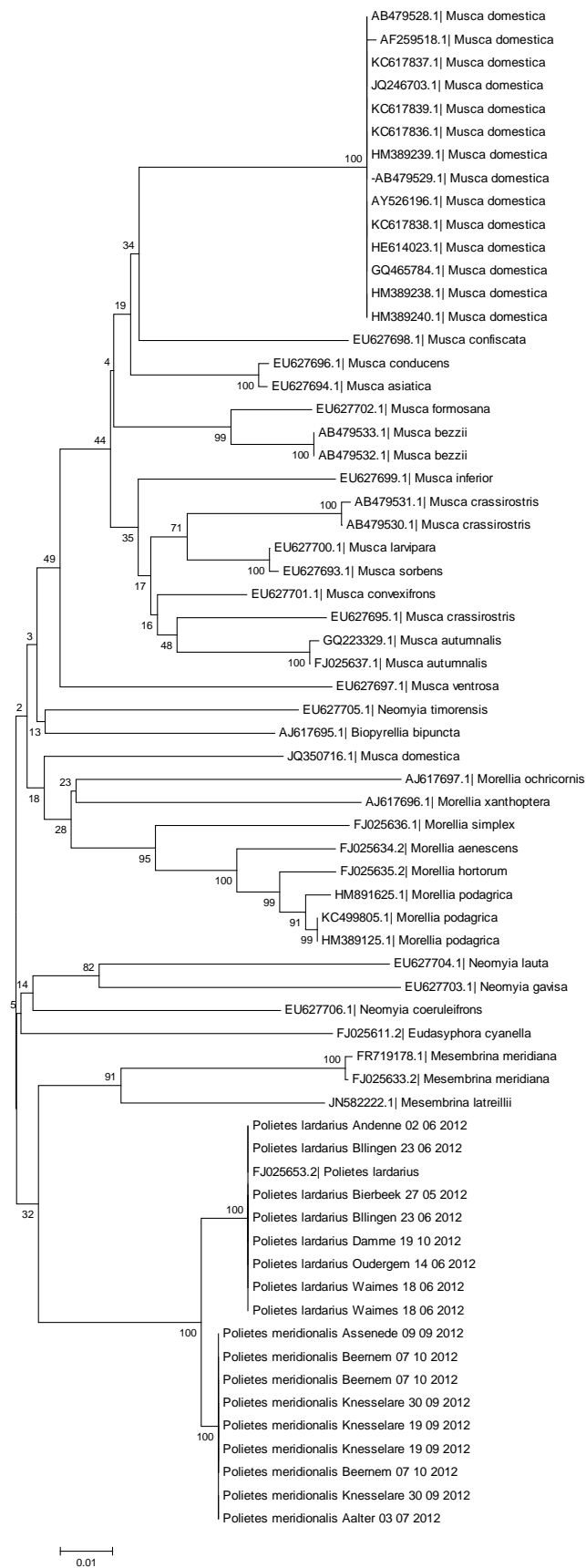


Fig. 5. Neighbour-joining tree of 68 COI sequences (658bp) from 31 Muscidae species. The tree is based on uncorrected p-distances and bootstrapping was done with 1000 replicates.

stored in 96% alcohol. The *P. lardarius* material in particular was collected on a rather broad geographic range, ranging from the Haute Fagnes in the East to Damme in the West of Belgium. Material of *P. meridionalis* was collected from a rather small area between Ghent and Brugge. Two legs were sampled from each specimen, put into a microtube and air-dried. Genomic DNA was extracted using the commercial NucleoSpin Tissue Kit (Macherey-Nagel, Germany) and following the protocol provided by the manufacturer. All fly specimens are kept in absolute ethanol as vouchers and are accessible in the collection of Dr. Grootaert (RBINS). A fragment of 658 bp of the 3'-end of the mitochondrial gene cytochrome oxidase subunit I (COI) was chosen to be amplified by PCR since it is standardly used for DNA barcoding in animals (HEBERT *et al.*, 2003). For each PCR reaction (total of 25 μ l), 2 μ l of the extracted DNA was used as template in a solution containing forward and reverse primers LCO1490 and HCO2198 (FOLMER *et al.*, 1994) at 0.2 μ M each, Platinum® Taq polymerase (Invitrogen) at 0.03 units/ μ l, dNTP at 0.2 mM each, Mg⁺⁺ at 1.5 mM and 1X PCR buffer (Invitrogen). PCR profile started with 3 minutes of initial denaturation at 94°C, followed by 40 cycles of denaturation at 94°C for 30 seconds, annealing at 50°C for 30 seconds and elongation at 72°C for 60 seconds. After these cycles, a final elongation step at 72°C was performed for 7 minutes. PCR products were cleaned up using ExoSAP (Fermentas). DNA sequencing was carried out on an ABI 3130xl automated capillary sequencer using BigDye v3.1. chemistry and following the manufacturer's instructions (Life Technologies, USA). DNA sequences were validated and corrected for sequencing errors. Assembly of the obtained DNA sequences were performed with BioEdit v.7.2. (HALL, 1999).

In addition, we selected 49 sequences from GenBank, representing 29 species of Muscidae and including one sequence of *Polietes lardarius* caught in Oxfordshire (UK) with accession number FJ025653. Sequences were aligned, pair-wise uncorrected p-distances were calculated and a neighbour-joining tree was constructed with complete deletion (i.e.

positions containing gaps and missing data were eliminated) using MEGA v5.01 (TAMURA *et al.*, 2011).

RESULTS OF DNA-BARCODING

The same haplotype was found in all samples of *Polietes meridionalis*. Another haplotype was found in all samples of *Polietes lardarius* (including the sequence from GenBank) except one, which was different from the other *Polietes lardarius* at a single site. Divergences between the haplotypes of both species corresponded to 9-10 substitutions (p-distances of 1.22%). In the neighbour-joining tree (Fig. 5), both species were found as sister-species in a clade supported with the maximum bootstrap value (100%). The single substitution (one basepair which has altered) differentiating the sequence of *Polietes lardarius* from its conspecifics is situated at a position for which data is missing in a few other sequences. This slight divergence is therefore not visible in the tree constructed with complete deletion (Fig. 5). Complete deletion is a method of building trees in which all characters for which data are lacking in one of the processed specimens are deleted in the others. In this way, a dataset is analysed for which all characters are known for all processed specimens.

Discussion

Polietes meridionalis and *P. lardarius* occur in Belgium for more than a century and nowadays both are widespread in the country. During the last four decades *P. meridionalis* has been increasingly recorded. Probably this southern species has slowly expanded its range northwards and has become more and more important in our region.

According to PERIS & LLORENTE (1963) *Polietes meridionalis* prefers more open and dry areas, while *P. lardarius* prefers more shaded and moist areas. We could not confirm this, on the contrary, we found that both species exploit a similar variety of situations and sometimes occur even syntopically.

We showed that there are some reliable morphological characteristics to distinguish the two species. In DNA barcoding, several arbitrary thresholds to distinguish species can be applied. For example, divergences of > 1% (RATNASINGHAM & HEBERT, 2007) or distances within each species that are smaller than distances between species can be used as a cut-off mark to distinguish different species (MEYER & PAULAY, 2005). As the divergence between *P. lardarius* and *P. meridionalis* is of 1.22%, which is higher than the intraspecific divergences (0%), both methods can be used to identify the two species.

However only a small geographic region has been sampled for *P. meridionalis*, and a somewhat wider geographic region for *P. lardarius*. In fact sampling of these two species is needed on a broader scale than Belgium. Furthermore, these criteria cannot be used solely as an argument for establishing the taxonomic validity of both species. But so far we have found consistent morphological and molecular differences between these two species which sometimes live syntopically, suggesting that they indeed represent different species.

RENAUD *et al.* (2012) assumed a recent divergence time for morphologically distinct species with distances to their nearest neighbours less than 2%. Similarly, it might be possible that *Polietes lardarius* and *Polietes meridionalis* have only recently diverged.

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