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A KEY FOR THE IDENTIFICATION OF LARVAE OF *ANOPLOPHORA CHINENSIS*, *ANOPLOPHORA GLABRIPENNIS* AND *PSACOTHEA HILARIS* (COLEOPTERA CERAMBYCIDAE LAMIINAE) IN EUROPE

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Pennacchio F., Sabbatini Peverieri G., Jucker C., Allegro G., Roversi P.F. – A key for the identification of larvae of *Anoplophora chinensis, Anoplophora glabripennis* and *Psacothea hilaris* (Coleoptera Cerambycidae Lamiinae) in Europe.

Anoplophora chinensis (Förster), *A. glabripennis* (Motschulsky) and *Psacothea hilaris* (Pascoe) (Coleoptera Cerambycidae Lamiinae) are longhorned beetles native to the far eastern regions of Asia and were recently accidentally introduced into Europe. The three exotic species are harmful insects to broadleaved plant species, and much attention is being paid to prevent further introductions and spread in the European Union. Severe phytosanitary measures are applied with the aim of eradicating outbreaks of the pests. Crucial for control is rapid identification of the longhorned species during phytosanitary inspections, both in entry ports and in the rest of the territory of the European Union. Taxonomic keys and descriptions of the adult morphology are available in the literature, but there are significant gaps in the taxonomy of larval morphology, and thus molecular analyses are required. During monitoring activities, a practical morphological taxonomic key would be a rapid and useful tool for species identification of the larvae. In the present work, a taxonomic key provided with detailed morphological pictures is proposed for the identification of the larvae of the three exotic species *A. chinensis*, *A. glabripennis* and *P. hilaris* among the closely related species of the native fauna of Europe.

KEY WORDS: exotic insect, quarantine pest, phytosanitary measures, xylophagous insect.

INTRODUCTION

Anoplophora chinensis (Förster), *Anoplophora glabripennis* (Motschulsky) and *Psacothea hilaris* (Pascoe) (Coleoptera Cerambycidae Lamiinae) are longhorned beetles native to the far eastern regions of Asia, and were recently accidentally introduced into Europe (EPPO, 2012). The larvae of these species develop in the phloem and xylem of host plants causing vegetative deterioration and structural weakness, which can lead to death of the trees.

A. chinensis and *A. glabripennis* are polyphagous species and are considered extremely harmful pests of a wide range of trees and shrubs of forest, urban, fruit and ornamental species; they are specified on the quarantine lists of Europe, USA and Canada (LINGAFELTER and HOEBEKE, 2002; EPPO, 2010; HAACK *et al.*, 2010). Specific phytosanitary regulations at different scales are in force in all countries of the European Union (EU), aimed at preventing the introduction and the spread of the pests and at eradicating any outbreaks; therefore, monitoring of the presence of these species in the territory of the EU is mandatory (EC, 2000; EU, 2012). Interceptions in EU entry ports and cases of infestations of both *Anoplophora* species have been reported for several countries (HAACK *et al.*, 2010; VAN DER GAAG *et al.*, 2010; EPPO, 2012). The monitoring activities involve inspecting plants in search of signs of beetle activity on infested plants or assessing the presence of adult beetles in the environment. The possibility of rapid identification of *Anoplophora* spp., confirming their presence in a given environment, is crucial to prevention of their introduction into new areas or for the success of the eradication program.

P. hilaris is also a polyphagous species, but with a more restricted host range linked to plants of the family Moraceae, mainly the genera *Morus* and *Ficus*. In the native area, this longhorned species is an important pest in sericulture (IBA, 1993). Although sericulture has almost disappeared in Europe, *Morus* trees are still grown for ornamental purposes, particularly in southern Europe. Moreover, around the Mediterranean Basin, *Ficus carica* is important for fig production, and many *Ficus* species are used for ornamental purposes.

Adults of *A. chinensis, A. glabripennis* and *P. hilaris* present characteristic habitus when compared with the native longhorned beetles of Europe and the adult morphology is well described in the literature (KUSAMA and TAKAKUWA, 1984; LINGAFELTER and HOEBEKE, 2002). In contrast, there are gaps in the morphological taxonomy based on the larvae, leading in most cases to the need for molecular analyses (KETHIDI *et al.*, 2003; STRANGI *et al.*, 2012). Therefore, classic taxonomical identification of the larvae would be a valid and rapid alternative in the diagnostic procedures and would be useful during phytosanitary inspections.

Milestones of the larval morphology of Cerambycidae are CRAIGHEAD (1915, 1916, 1923), DUFFY (1953, 1968), NAKAMURA and KOJIMA (1981), ŠVÁCHA and DANILEVSKY (1986, 1987, 1988) and CHEREPANOV (1990, 1991a, 1991b), while works concerning the description of larval morphology of *Anoplophora* species are LIEU (1945),

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CAVEY *et al.* (1998) and LINGAFELTER and HOEBEKE (2002). However, taxonomic morphological keys for the larvae of *A. chinensis*, *A. glabripennis* and *P. hilaris*, which would allow their identification among the larvae of the native European longhorned fauna, are not available. Therefore, we present here a key provided with detailed pictures of larval morphology for the identification of larvae of *A. chinensis*, *A. glabripennis* and *P. hilaris* within the context of the phytosanitary measures adopted in Europe.

MATERIAL AND METHODS

In the present study, the work by LÖBL and SMETANA (2010) was adopted for the systematic arrangement while ŠVÁCHA and DANILEVSKY (1986) was used for the nomenclature of larval morphology. The biological material was prepared for analysis using methods proposed by ŠVÁCHA and DANILEVSKY (1986): larvae were narcotized with ethyl acetate, followed by immersion in boiling water for a period of time varying from 2 - 20 seconds depending on the body size of the specimens. To avoid osmotic deformations of the body, some small holes were drilled in the cuticle with the aid of entomological needles. Prepared specimens were stored in Pampel's fluid or acid alcohol (ŠVÁCHA and DANILEVSKY, 1986; STEHR, 1987). Most of the specimens were collected in the field during entomological surveys and prepared as described above, but some of the material was obtained from the entomological collection of the CRA-ABP in Florence.

RESULTS

The general aspects of the larvae of *A. chinensis*, *A. glabripenni*s and *P. hilaris* are common to the family Cerambycidae (Figs. I and II): larvae are elongate, cylindrical, fleshy, cream-colored; the head is prognathous and usually retracted into the prothorax; the prothorax is always larger than the meso- and metathorax and abdomen; the pronotum is more or less partially sclerotized and often provided with a pronotal pigmented plate; the abdomen has 10 visible segments; sprialces are present on the mesothorax and on abdominal segments I-VIII; like some native European species, *A. chinensis*, *A. glabripenni*s and *P. hilaris* have relatively large larvae when mature, up to 50 mm in body lenght or slightly more (LIEU, 1945; STEHR, 1987; LINGAFELTER and HOEBEKE, 2002; CAVEY *et al.*, 2003; HAACK *et al.*, 2010). These common general aspects make species identification of the larvae of the three exotic beetles difficult and they can be confused with some native European longhorned beetles.

The European fauna lists approximately 680 species in the family Cerambycidae, grouped into 8 sub-families (FE, 2012). *A. chinensis*, *A glabripennis* and *P. hilari*s are cerambycid species belonging to the tribe Monochamini of the sub-family Lamiinae. Among the European fauna, there are about 340 species listed in this sub-family, but only one genus of the native species represents the tribe Monochamini (*Monochamus* Dejean). The sub-family Lamiinae contains some species with features and body size similar to those of the three exotic species considered here. Hence, they can likely be mistaken for example for *Morimus asper* (Sulzer) and *Lamia textor* L. (both of the tribe Lamiini), the species in the genus *Monochamus* (tribe Monochamini), and the species *Saperda carcharias* L. (tribe Saperdini).

TAXONOMIC KEY FOR IDENTIFICATION OF THE EXOTIC SPECIES *A. CHINENSI*S, *A. GLABRIPENNIS* AND *P. HILARIS* AMONG THE CERAMBYCIDAE OF THE EUROPEAN FAUNA (BASED ON LAST INSTARS LARVAE)

1 - Legs present, 4 jointed (excluding coxa) (Fig. III)
sub-fam. Prioninae Latreille
sub-fam. Parandrinae Latreille
sub-fam. Vesperinae Mulsant
sub-fam. Lepturinae Latreille
sub-fam. Necydalinae Linnaeus
sub-fam. Spondylidinae Audinet-Serville
sub-fam. Cerambycinae Latreille (*pars*)
- Legs absent (sub-fam. Cerambycinae (*pars*) and sub-fam. Lamiinae Latreille) 2

2 - Clypeus very narrow, with only slender basal arms reaching to mandibular articulations (Fig. IV). Mandibular apex and dorsal angle more or less lacking; mandible short, apically rounded, spoon-like (Fig. V)
sub-fam. Cerambycinae Latreille
- Clypeus more or less trapezoidal, filling entire space between dorsal mandibular articulations (Fig. VI). Mandibles not rounded, with distinct apex and more or less distinct dorsal angle (Fig. VII) (sub-fam. Lamiinae Latreille) 3

3 - Anal pore transverse (Fig. VIII) tribe Lamiini Latreille
- Anal pore triradiate (one ventral and two lateral rays) (Fig. IX); the ventral ray can be shorter in some species (Figs. X and XI) 4

4 - Pronotal shield and dorsal ambulatory ampullae with dark spinule visible under a low magnification (Figs. XII and XIII) tribe Saperdini Mulsant
- Pronotal shield and dorsal ambulatory ampullae with very minute spinule visible under high magnification. In some tribes (Lamiini, Monochamini, *etc.*) the pronotal shield under low magnification appears as a dark uniform plate, provided with small depigmented rounded areas, more or less joined (Figs. XIV, XV and XVI). Dorsal ambulatory ampullae with different features (Figs. XVII, XVIII and XIX) and never provided with visible spinule under low magnification. In some tribes a distinct pronotal shield is lacking (Fig. XX) 5

5 - Dorsal ambulatory ampullae granular, build up by small granules in distinct transvere rows or in elongate oval clusters formed by large joined granules (Figs. XVII and XVIII) 6
- Dorsal ambulatory ampullae not granular, but with small spinule (Fig. XIX) tribe Acanthocinini Blanchard

6 - Dorsal ambulatory ampullae medially with large granules in 4 distinct transverse rows (Fig. XVII). Body size of the last instars larvae generally more than 40 mm (tribe Monochamini Gistel) 7
- Dorsal ambulatory ampullae with different aspect, granules in less than 4 rows or in elongated oval clusters formed by large joined granules (Fig. XVIII). Last instars larvae smaller than 35 mm
For example: tribe Pteropliini Thomson
tribe Acanthoderini Thomson
tribe Mesosini Mulsant
and other tribes not Monochamini

7 - Abdominal epipleurum of the segments III-IX protu-

berant (Fig. XXI). Anal pore with the ventral ray distinctly shorter than the two rays (Figs. X and XI) 8
- Abdominal epipleurum protuberant only on the segments VII-IX. Anal pore with the ventral and two lateral rays of the same length; in some cases, the ventral ray is slightly shorter (Fig. IX) 9

8 - Abdominal segments provided, laterally from the dorsal ambulatory ampullae to the epipleurum, with a number of setae (generally more than 100 for each side), some of them being up to 3 - 4 times as long as the major diameter of the corresponding abdominal spiracle (Fig. XXII). Setae of abdominal segments IX and X numerous and very long (Fig. X). Pleural tubercles with 2 small sclerotized dots (Fig. XXIV). Species developing on conifers of the genera *Pinus, Picea* and *Abies*
genus *Monochamus* Dejean
- Abdominal segments provided, laterally from the dorsal ambulatory ampullae to the epipleurum, with a smaller number of setae (generally less than 60 for each side), with some of them shorter then approximately 2 times the major diameter of the corresponding abdominal spiracle (Fig. XXIII). Setae of abdominal segments IX and X less numerous and short (Fig. XI). Pleural tubercles without sclerotized dots (Fig. XXV)
Psacothea hilaris (Pascoe)

9 - Although, the species separation through larval morphology can be quite difficult, the following diagnostic aspects can be proposed:
- A distinct pigmented band is present anterior to the pronotal shield; typical pronotum as in Fig. XIV
Anoplophora chinensis (Förster)
- Anterior to the pronotal shield, the band is less observable due to less pigmentation; typical pronotum as in Fig. XV *Anoplophora glabripennis* (Motschulsky)

FURTHER NOTES ON LARVAL MORPHOLOGICAL DIFFERENCES AMONG THE EXOTIC SPECIES *A. CHINENSIS, A. GLABRIPENNIS*, *P. HILARIS* AND OTHER LONGHORNED BEETLES OF THE TRIBE LAMIINI NATIVE TO EUROPE.

There are additional characters in the larval morphology which can be considered in the identification of the exotic species *A. chinensis*, *A. glabripennis* and *P. hilaris* in cases when the body parts of the larvae are not entirely observable or are missing, *i.e.* if parts of the larval body are lost during extraction from the infested plants. However, for species identification in these cases, at least the whole anterior part of the larvae up to abdominal segment III must be available for observation. In such cases, where the anal pore structure is not observable, the key proposed above can be used to sequentially separate all species of the tribes different from Lamiini and the three exotic species, since the considered diagnostic characters after step 3 of the key concern the anterior part of the larval body. Once all other tribes can be excluded, following characters can be used to separate the European species of

Fig. I – Larvae of Lamiinae: *Anoplophora chinensis* (Förster), lateral view.

Fig. II – Larvae of Lamiinae: *Anoplophora chinensis* (Förster), dorsal view.

Fig. III – Larvae of Prioninae: legs in *Ergates faber* (L.).

Fig. IV – Larvae of Cerambycinae: *Plagionotus arcuatus* (L.), the arrow indicates the clypeus.

Fig. V – Larvae of Cerambycinae: *Plagionotus arcuatus* (L.), the arrow indicates the apical margin of the mandibles.

Fig. VI – Larvae of Cerambycinae: *Monochamus galloprovincialis* Olivier, the arrow indicates the clypeus.

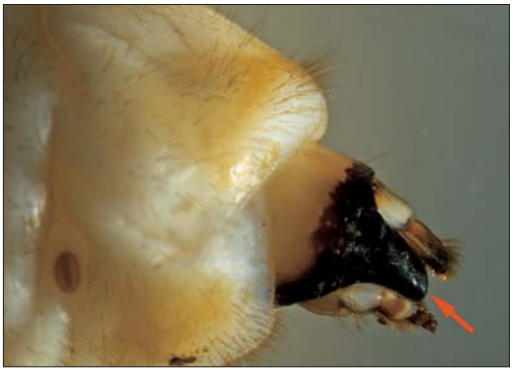

Fig. VII – Larvae of Lamiinae: *Aegomorphus clavipes* (Schrank), the arrow indicates the apex of the mandibles.

Fig. VIII – Larvae of Lamiinae: *Morimus asper* (Sulzer), anal pore.

Fig. IX – Larvae of Lamiinae: *Anoplophora chinensis* (Förster), anal pore.

Fig. X – Larvae of Lamiinae: *Monochamus galloprovincialis* Olivier, anal pore.

Fig. XI – Larvae of Lamiinae: *Psacothea hilaris* (Pascoe), anal pore.

Fig. XII – Larvae of Lamiinae: *Saperda carcharias* L., pronotal shield.

Fig. XIII – Larvae of Lamiinae: *Saperda carcharias* L., dorsal ambulatory ampullae of the II abdominal segment.

Fig. XIV – Larvae of Lamiinae: *Anoplophora chinensis* (Förster), pronotal shield.

Fig. XV – Larvae of Lamiinae: *Anoplophora glabripennis* (Motschulsky), pronotal shield.

Fig. XVI – Larvae of Lamiinae: *Morimus asper* (Sulzer), pronotal shield.

Fig. XVII – Larvae of Lamiinae: *Anoplophora glabripennis* (Motschulsky), dorsal ambulatory ampullae of the I abdominal segment.

Fig. XVIII – Larvae of Lamiinae: *Aegomorphus clavipes* (Schrank), dorsal ambulatory ampullae of the I-III abdominal segments.

Fig. XIX – Larvae of Lamiinae: *Acanthocinus griseus* (Fabricius), dorsal ambulatory ampullae of the IV abdominal segment.

Fig. XX – Larvae of Lamiinae: *Aegomorphus clavipes* (Schrank), pronotum without a distinctive shield.

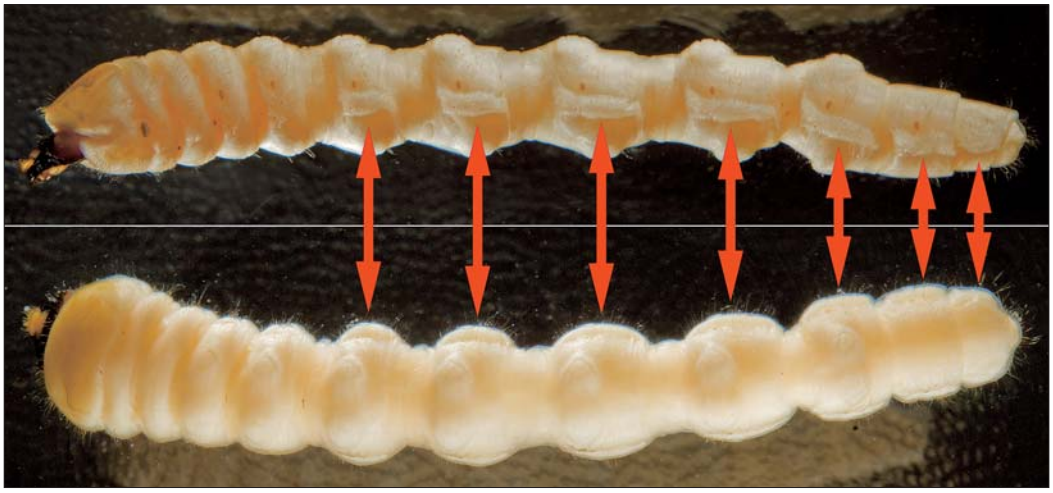

Fig. XXI – Larvae of Lamiinae: *Monochamus galloprovincialis* Olivier, lateral and dorsal view, protuberant epipleurum of the III - IX abdominal segments (arrows).

Fig. XXII – Larvae of Lamiinae: *Monochamus galloprovincialis* Olivier, epipleurum of the III and IV abdominal segment.

Fig. XXIII – Larvae of Lamiinae: *Psacothea hilaris* (Pascoe), epipleurum of the III and IV abdominal segment.

the tribe Lamiini and the species *A. chinensis*, *A. glabripennis* and *P. hilaris*. Native European species of the tribe Lamiini belong to 5 genera[1]: *Morimus* (only one species[2]: *M. asper* with several sub-species), *Lamia* (only one species: *L. textor*), *Herophila, Dorcadion* and *Neodorcadion* (FE, 2012). Species belonging to these genera can be separated from *A. chinensis*, *A. glabripennis* and *P. hilaris* by the aspects described below. Regarding *Anoplophora* spp., the pigmentation of the lateral parts of the head capsule (pleurostoma and gena) is much more expanded posteriorly in larvae of *M. asper* (Fig. XXVI) than in *Anoplophora* spp. (Fig. XXVII). The pleural tubercles in *Anoplophora* spp. are provided with 2 – 3 (some times 4) setae (Fig. XXVIII), while in *M. asper* there are generally 5 - 8 (Fig. XXIX). Moreover, in *M. asper* the setae on the

[1] An additional genus is *Taeniotes* Serville, but it is represented by only one species, *T. scalatus* (Gmelin), present in Europe exclusively in the Azores archipelago.

[2] A second species of the genus *Morimus* is *M. orientalis* Reitter, but its natural area in Europe is restricted to the European part of Turkey.

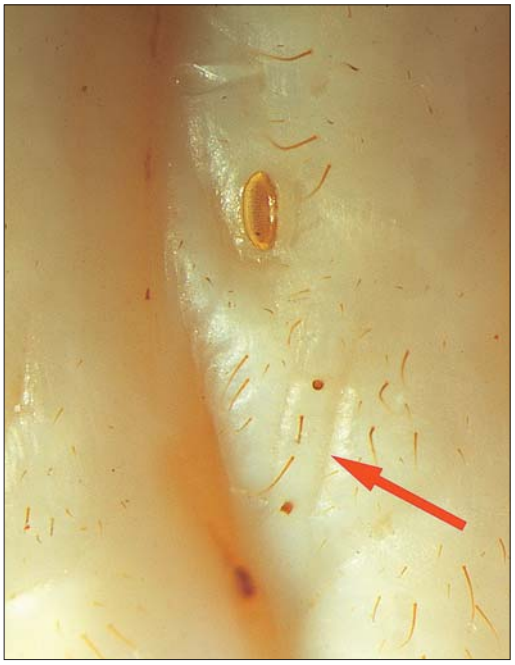

Fig. XXIV – Larvae of Lamiinae: *Monochamus galloprovincialis* Olivier, the arrow indicates the pleural tubercle of the I abdominal segment.

Fig. XXV – Larvae of Lamiinae: *Psacothea hilaris* (Pascoe), the arrow indicates the pleural tubercle of the I abdominal segment.

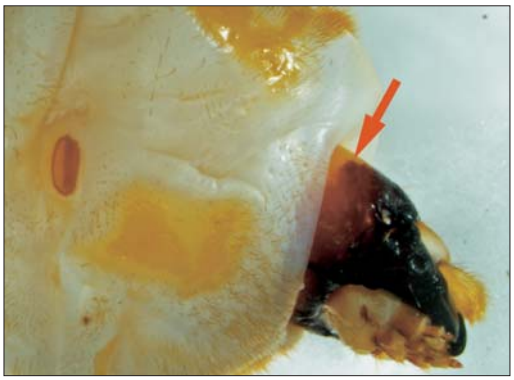

Fig. XXVI – Larvae of Lamiinae: *Morimus asper* (Sulzer), the arrow indicates the backwards expansion of the pigmentation of the pleurostoma and gena.

Fig. XXVII – Larvae of Lamiinae: *Anoplophora glabripennis* (Motschulsky), the arrow indicates the pigmentation not expanded backwards of pleurostoma and gena.

lateral parts of the abdominal segments III-VIII (from the ambulatory ampullae to the epiplerum) are always more numerous (approximately 180-220), while in *Anoplophora* spp. there are generally not more than 130 setae. The larvae of *L. textor* present a higher number of setae (180-240) respect to *Anoplophora* spp. on the lateral parts of the abdominal segments, and the epipleurum of the abdominal segments is distinctly protuberant in segments III-IX, while in *Anoplophora* spp. only in segments VII-IX.

In a similar way, misunderstandings can occur among *P. hilaris* and species of the tribe Lamiini. In *M. asper* the epiplerum is protuberant starting from abdominal segment VII, while in *P. hilaris* starting from segment III. In *M. asper* there are two sclerotized dots on the epipleural tubercles (Fig. XXIX), which are absent in *P. hilaris* (Fig. XXV). Moreover, in *M. asper* the number of setae present on the area between the dorsal ambulatory ampullae and the epipleurum is higher than in *P. hilaris* (< 80). Differences between *L. textor* and *P. hilaris* are that in the former the two sclerotized dots on the epipleural tubercles are present, and laterally the number of setae between the dorsal ambulatory ampullae and the epipleurum is higher in *L. textor*.

All three exotic species considered here can be separated from the other European species of the tribe Lamiini, *i.e.* the genera *Herophila*, *Dorcadion* and *Neodorcadion*, since those species are in general much more smaller than *Anoplophora* spp. and *P. hilaris* and the last instars larvae are generally less than 35 mm in body length. Moreover, the genera *Dorcadion* and *Neodorcadion* include species which develop only in roots of herbaceous plants.

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Fig. XXVIII – Larvae of Lamiinae: *Anoplophora glabripennis* (Motschulsky), the arrow indicates the pleural tubercle of the I abdominal segment

Fig. XXIX – Larvae of Lamiinae: *Morimus asper* (Sulzer), the arrow indicates the pleural tubercle of the I abdominal segment.

glabripennis, and to Matteo Maspero of the Fondazione Minoprio (Vertemate con Minoprio, Italy) and Daniela Lupi of the University of Milano (Milan, Italy) for providing larvae of *A. chinensis* and *P. hilaris* respectively. We are grateful to Paola Furlan for laboratory assistance and to the Italian Regional Plant Protection Services of Lazio, Lombardy and Veneto for supporting during field work. This work was carried out within the activities of the EUPHRESCO funded project ANOPLORISK and the STRATECO project of the Italian Ministry of Agricultural, Food and Forestry Policies (DM 30290/7303/09).

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