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Standard Paper

Catillaria flexuosa (*Catillariaceae*), a new lichen species described from the Netherlands

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Abstract

A new lichen species is described from specimens growing on *Fraxinus* trees north of Eindhoven (the Netherlands). Morphological and genetic studies suggest that the new species belongs in the genus *Catillaria*, and the name *Catillaria flexuosa* is proposed because of its flexuose apothecia. The new species is characterized by the relatively large apothecia (up to 0.9 mm diam.) and relatively thick, knobby to ±subsquamulose, greenish, thallus. Due to their similar morphological features, *C. flexuosa* can be easily confused with *Catillaria chalybeia*, *C. fungoides* or *C. nigroclavata*, so it is therefore compared with these species. In addition, *Arthonia epiphyscia* is reported being a very rare species in the Netherlands.

Key words: Ascomycota, Benelux, *Leprocaulales*, mtSSU, taxonomy, 28S nrDNA

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Introduction

A long and intensive study of lichens in the south-east part of the Noord-Brabant Province (the Netherlands) resulted in multiple interesting taxonomic novelties (e.g. van den Boom 2004, 2015; van den Boom & van den Boom 2009). Recently, a collection was found growing on a medium-sized trunk of *Fraxinus excelsior*. It was thought to be a species of *Catillaria* A. Massal. because it presented paraphyses with black tips, a feature typically found in this genus, as well as relatively small and hyaline 1-septate ascospores.

The first genetic data from the type species of *Catillaria*, *C. chalybeia* (Borr.) A. Massal., was provided by Bendiksby & Timdal (2013), who showed that this species is closely related to the genera *Solenopsora* A. Massal. (type species *S. requienii* A. Massal.) and *Lopadium* Körb. (type species *L. pezizoideum* (Ach.) Körb.), forming a monophyletic clade outside the main group of *Lecanorales* Nannf. Miadlikowska *et al.* (2014) identified this clade as *Leprocaulales* Lendemer & B. P. Hodk., an invalid name due to the lack of a description or diagnosis in Lendemer & Hodkinson (2013), where this clade was shown to include also samples identified as *Halecania alpivaga* (Th. Fr.) M. Mayrhofer (the type species of *Halecania* M. Mayrhofer) and *Speerschneidera euploca* (Tuck.) Trevis. (the type species of *Speerschneidera* Trevis.).

According to the genetic results obtained by several authors (Andersen & Ekman 2005; Reese Næsborg *et al.* 2007; Ekman *et al.* 2008; Lee *et al.* 2008; Kistenich *et al.* 2018), the traditional concept of the genus *Catillaria* is not monophyletic. Several species belong in unrelated families according to Miadlikowska *et al.* (2014); namely *Catillaria alba* Coppins & Vězda, *C. aphana* (Nyl.) Coppins, *C. corymbosa* (Hue) M. Lamb, *C. croatica* Zahlbr. and *C. scotinoides* (Nyl.) Coppins in *Ramalinaceae*, *C. contristans* (Nyl.) Zahlbr. in *Pilocarpaceae*, *C. erysiboides* (Nyl.) Th. Fr. in *Psilolechiaceae*, and *C. modesta* (Müll. Arg.) Coppins in *Lecideaceae*. Kelly *et al.* (2011) produced molecular data from specimens identified as *C. nigroclavata* and later Guttová *et al.* (2014) obtained data from other samples identified as *C. lenticularis*, but they could not provide any hypothesis about their placement at the family level.

Three species of *Catillaria* have been described from Australia and Tasmania recently and from the type locality only: *Catillaria austrolittoralis* Kantvilas & van den Boom (Kantvilas & van den Boom 2013) and *C. laevigata* P. M. McCarthy & Elix (Elix & McCarthy 2018), two saxicolous species with a rimose or areolate thallus, containing argopsin or pannarin; *C. gerroana* P. M. McCarthy & Elix (McCarthy & Elix 2017), which lacks these chemical compounds and has ascospores of (9–)11.5(–15) × (3.5–)4.5(–5.5) µm. Another saxicolous species, *C. ulleungdoensis* S. Y. Kondr. *et al.*, was recently described from South Korea; it has a weakly developed thallus and rather small apothecia (0.15–0.3(–0.55) mm diam.) (Kondratyuk *et al.* 2016). *Catillaria patteeana* D. P. Waters & Lendemer has brown apothecia with an opaque white to light brown margin and is sorediate (Waters & Lendemer 2019). Two corticolous species, *C. praedicta* Tretiach & Hafellner and *C. subpraedicta* M. Brand & van den Boom, known from Mediterranean areas and Lanzarote (Canary Islands) respectively, have multispored asci.

In the present work, the taxonomic identity of the new samples found with *Fraxinus* in Noord-Brabant is resolved.

Materials and Methods

Morphological study and literature

All specimens collected were studied by conventional macro- and microscopical techniques after mounting hand-cut sections of the

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Table 1. Species names, voucher specimens and GenBank Accession numbers of sequences of *Catillaria* and related taxa used in the phylogenetic analyses. Samples in bold were sequenced in the present study. Sequences were obtained mainly from Gaya *et al.* (2012), Lendemer & Hodkinson (2013), Guttová *et al.* (2014) and Prieto & Wedin (2016).

		Accession numbers				
Species name	Voucher	ITS	LSU	mtSSU	*Mcm7*	*Tub2*
Acolium inquinans	*Wedin* 6352 (UPS)	AY450583	AY453639	AY143404	JX000161	-
Acroscyphus sphaerophoroides	*Obermayer* 6077 (UPS)	KX512898	-	KX512984	KX529029	-
Allocalicium adaequatum	*Spribille* 14143 (UPS)	KX512906	KX512859	KX512986	-	KX528996
Amandinea punctata	*Wedin* 2/3/96 (UPS) / AFTOL-ID 1306	KX512899	DQ986756	DQ986874	KX529025	-
Anaptychia ciliaris	*Wedin* 6429 (UPS)	AY143391	KX512894	-	KX529054	-
Athallia scopularis	*Søchting* 7521 (C), USE197	KC179340	KC179150	KC179480	-	-
Baculifera remensa	*Prieto* (S)	-	KX512881	KX512962	-	-
Bryobilimbia diapensiae	*Arup* L04400	HQ650660	HQ660539	HQ660564	-	-
Buellia disciformis	*Wedin* 6155 (BM)	AY143392	JX000082	AY143401	JX000152	-
Calicium viride	*Wedin* 24/4 2000 / *Prieto* 3015 (S)	HQ650703	AY340538	AY143402	JX000153	KX529013
Calogaya decipiens	USE15, 1995 *Søchting* (C) / *Arup* L06187 / JV7964	KC179344	KC179167	KC179498	-	KY748807
Caloplaca cerina	*Elvebakk* 03-084 (TROM)	KC179425	KC179168	KC179499	-	-
Catillaria chalybeia	***P.v.d. Boom* 59147 (hb. P.v.d. Boom)**	**MT248987**	**MT248985**	-	-	-
C. chalybeia	***P.v.d. Boom* 59646 (hb. P.v.d. Boom)**	**MW045826**	**MW045829**	**MW077133**	-	-
C. chalybeia	*R. Haugan* 7947 / 155291 (O-L)	KF360370	KF360447	-	-	-
C. flexuosa	***P.v.d. Boom* 58587 (holotype, B)**	**MT248986**	**MT248984**	-	-	-
C. flexuosa	***P.v.d. Boom* 59636 (hb. P.v.d. Boom)**	**MW045827**	**MW045830**	-	**MW049361**	-
C. lenticularis	*Fackovcova* 21-Oct-2011 (SAV)	KF689898	-	-	-	-
C. nigroclavata	EDNA09-02107 (E)	FR799313	-	-	-	-
C. nigroclavata	EDNA09-02108 (E)	FR799312	-	-	-	-
C. nigroclavata	G.M. 2015-07-09.5 (LUX)	MT912697	-	-	-	-
***Catillaria* sp.**	***P.v.d. Boom* 55102 (hb. P.v.d. Boom)**	**MW045825**	**MW045828**	-	-	-
Culbersonia nubila	ALV14224	-	MH121319	-	-	-
Cyphelium tigillare	*Prieto* 3038 (S)	JX000104	JX000088	JX000123	JX000162	KX529002
Dimelaena oreina	*Lendemer* 4193 (S)	KX512922	KX512867	KX512976	KX529036	KX528999
Diplotomma alboatrum	*Prieto* 3034 (S)	KX512924	KX512877	KX512966	KX529043	KX529007
Dirinaria applanata	*Seaward* 109735 (S)	-	KX512856	DQ972983	-	-
Fulgensia fulgens	*Gaya* 39 (BCN)	JQ301671	JQ301567	JQ301503	-	-
Halecania alpivaga	195847 (O-L)	KY266832	-	AY756368	-	-
Heterodermia speciosa	*Wetmore* 88030 (S)	KX512927	JX000089	KX512975	JX000166	KX529000
Leprocaulon adhaerens	NY351, *Lendemer* 13546 (NY)	KC184100	-	-	-	-
L. adhaerens	NY598, *Morse* 16380 (NY)	KC184101	-	-	-	-
L. americanum	NY184, *Knudsen* 9605 (NY)	KC184112	-	KC183999	-	-
L. americanum	NY185, *Lendemer* 11445 (NY)	KC184111	-	-	-	-
L. calcicola	E2080	KX674677	-	-	-	-
L. calcicola	E2117, *C.J.B. Hitch* (type, NMW)	NR_158498	-	-	-	-
L. knudsenii	NY62, *Lendemer* 11476 (NY)	KC184115	-	-	-	-
L. knudsenii	NY702, *Knudsen* 9256 (type, NY)	KC184076	-	-	-	-
L. microscopicum	*Ekman* 3685 (BG)	AF517921	-	-	-	-

(*Continued*)

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Table 1. (*Continued*)

Species name	Voucher	Accession numbers				
		ITS	LSU	mtSSU	*Mcm7*	*Tub2*
L. quisquiliare	98GB04	MN959918	-	-	-	-
L. quisquiliare	L-200884 (O)	MK812636	-	-	-	-
L. quisquiliare	NY1261, *Buck* 55960 (NY)	-	KJ766593	KC183993	-	-
L. santamonicae	NY1264, *Knudsen* 12058 (NY)	KC184105	-	KC183995	-	-
L. santamonicae	NY565, *Lendemer* 19660 (NY)	KC184108	-	-	-	-
L. terricola	NY355, *Knudsen* 2659.2 (NY)	KC184102	-	KC184017		
L. terricola	NY655, *Knudsen* 9608 (NY)	KC184103	-	-	-	-
L. textum	NY57, *Lendemer* 11500 (NY)	KC184113	-	-	-	-
Letrouitia domingensis	AFTOL-ID 102	HQ650700	AY584648	AY584619	-	-
Megalospora sulphurata	*Kantvilas* 376/09 (F)	JQ693555	JQ693538	JQ693518	-	-
Phaeophyscia ciliata	*Prieto* (S)	KX512929	KX512886	KX512958	KX529051	KX529012
Physcia tenella	*Odelvik* & *Hellström* 0827 (S)	KX512932	KX512869	KX512974	KX529038	-
Physconia muscigena	17040 (BCN-Lich)	GU247161	-	GU247195	-	-
Polycauliona polycarpa	USE599, *Søchting* 10507 (C)	KC179389	KC179222	KC179558	-	-
Pseudothelomma ocellatum	*Tehler* 8063 (S)	KX512934	KX512862	KX512957	KX529028	KX529019
Pyxine sorediata	*Wetmore* 91254 (S)	KX512937	JX000093	KX512973	JX000179	KX529001
Rusavskia elegans	MP78, *Odelvik* 04532 (S)	KX512947	KX512896	KX512948	KX529056	KX529024
Solenopsora candicans	*D. Ertz* 7604 (DUKE) / *Guttova* (SAV)	KF689797	KJ766660	KJ766493	KF689899	-
S. cesatii	cesatii_19_FR / cesatii_10_FR (SAV)	KF689850	-	-	KF689933	-
S. cesatii	cesatii_9_SK (SAV)	KF689849	-	-	-	-
S. chihuahuana	BC51, L-15843b (O)	MN148640	-	-	-	-
S. crenata	BC67, *Nash*-38664b (ASU)	MN148641	-	-	-	-
S. grisea	grisea_7_IT (SAV)	KF689874	-	-	-	-
S. grisea	grisea_9_AL (BP)	KF689875	-	-	-	-
S. grisea subsp. *grisea*	grisea_4_MK / grisea_11_ME (SAV)	KF689871	-	-	KF689970	-
S. holophaea	1865	AM292708	-	-	-	-
S. holophaea	holophaea_1_FR (SAV)	KF689876	-	-	-	-
S. liparina	liparina_1_FR (SAV)	KF689877	-	-	-	-
S. liparina	liparina_2_FR (SAV)	KF689878	-	-	-	-
S. liparina	liparina_3_CZ/ 2014_6l (SAV)	KF689879	-	-	MN166068	-
S. marina	marina_5_TR (SAV)	KF689884	-	-	-	-
S. marina	*Slovak* & *Kucera* 27-May-2012 (SAV)	KF689885	-	-	KF689981	-
S. olivacea	olbiensis_1_IT (SAV)	KF689886	-	-	-	-
S. olivacea	olivacea_2_HR (SAV)	KF689888	-	-	-	-
S. olivacea	*Timdal* 21-Jul-2010 (O)	KF689889	-	-	KF689990	-
S. vulturiensis	0290344 (M)	MH269330	-	-	-	-
S. vulturiensis	98GB03	MN959915	-	-	-	-
S. vulturiensis	vulturiensis_3_FR (SAV)	KF689896	-	-	-	-
S. vulturiensis	vulturiensis_4_FR (SAV)	KF689897	-	-	KF689998	-
Speerschneidera euploca	*Egan* 14906 (F)	-	AY300862	AY300912	-	-
Teloschistes flavicans	*E. Gaya* 02.26.10-8 & *F. Lutzoni* (DUKE) / *Frödén* 1624 (LD)	KT291472	KC179255	KC179594	-	-

(*Continued*)

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Table 1. (*Continued*)

Species name	Voucher	Accession numbers				
		ITS	LSU	mtSSU	*Mcm7*	*Tub2*
Tetramelas pulverulentus	*Nordin* 6368 (UPS)	KX512940	KX512860	KX512983	-	KX528990
Texosporium sancti-jacobi	*Rosentreter & De Bolt* 6514 (UPS)	KX512941	KX512863	JX000132	JX000187	KX528994
Thelomma mammosum	*Tibell* 23775 (UPS)	KX512942	KX512888	KX512954	KX529065	KX529016
Tholurna dissimilis	*Wedin* 6330 (UPS)	AY143397	KX512893	AY143407	KX529053	KX528992
Tornabea scutellifera	*Tibell* 23833 (UPS)	KX512946	KX512873	KX512970	KX529042	-
Xanthocarpia ochracea	*Gaya* 21, *P. Navarro-Rosines* (BCN)	-	JQ301558	JQ301494	-	-
Xanthodactylon flammeum	36/1940 (HSBG)	EU681315	-	EU680897	-	-
Xanthomendoza mendozae	*Søchting* 10209 (C)	KC179138	KC179281	KC179620	-	-
Xanthoria parietina	*Gaya* 8 (BCN)	JQ301691	JQ301589	-	-	-

material in tap water. Amyloid reactions were tested using Lugol's iodine solution (K/I). Accompanying specimens were identified. Photographs of habitus and microscopic characters are provided for the new species. Voucher specimens are kept in the herbarium of the first author and some specimens, including the holotype specimen, have been deposited in the herbarium of the Botanischer Garten und Botanisches Museum Berlin-Dahlem (B). Differences between corticolous species of *Catillaria* s. str. are summarized in Table 2 (data obtained from Kilias (1981), Tretiach & Hafellner (1998), van den Boom & Etayo (2001), Smith *et al.* (2009), van den Boom (2010); and from corticolous *Catillaria chalybeia* specimens in this study).

Phylogenetic studies

Total DNA was extracted from dry specimens employing a modified protocol based on Murray & Thompson (1980). PCR reactions (Mullis & Faloona 1987) included 35 cycles with an annealing temperature of 54 °C. The primers ITS1F and ITS4 (White *et al.* 1990; Gardes & Bruns 1993) were employed to amplify the ITS rDNA region (ITS), LR0R and LR5 (Vilgalys & Hester 1990; Cubeta *et al.* 1991) were used for the 28S rDNA region (LSU), mrSSU1 and mrSSU3R for the mitochondrial small ribosomal subunit (mtSSU), and finally MCM7-709for and MCM7-1348rev for the DNA replication licensing factor (*Mcm7*). PCR products were checked in 1% agarose gels and positive reactions were sequenced with one or both PCR primers. Chromatograms were checked, searching for putative reading errors, and these were corrected. BLAST (Altschul *et al.* 1990) was used to select the most closely related sequences from the International Nucleotide Sequence Database Collaboration public database (INSDC; Cochrane *et al.* 2011). Two independent datasets were built. The first alignment comprised sequence data of ITS2, LSU, mtSSU and *Mcm7* (exons only) from the samples analyzed. Also included were homologous sequences from other species in the *Leprocaulaceae* and *Catillariaceae* families retrieved from public databases, and representatives from the orders *Caliciales* and *Teloschistales*, using *Bryobilimbia diapensiae* (Th. Fr.) Fryday *et al.* as outgroup because of its external position relative to these clades. The purpose of this analysis was to ascertain whether or not the newly generated sequences belong in *Leprocaulales*, as several species of *Catillaria* have been combined into different families and orders in the past. In addition, *tub2* sequences available from the species in the alignment were incorporated, making a total of five partitions. Sequences (Table 1) came mainly from Gaya *et al.* (2012), Lendemer & Hodkinson (2013), Guttová *et al.* (2014) and Prieto & Wedin (2016). Each partition was aligned in MEGA 5.0 (Tamura *et al.* 2011) with its Clustal W application and then corrected manually. The final alignment included 126/266 (ITS2, $n = 59$), 251/652 (LSU, $n = 44$), 339/602 (mtSSU, $n = 43$), 217/443 (*Mcm7*, $n = 27$) and 217/624 (*tub2*, $n = 15$) variable/total sites. The aligned partitions were loaded in MrBayes v.3.2.6 (Ronquist *et al.* 2012), where a Bayesian analysis was performed (data partitioned, GTR + G model for all partitions, two simultaneous runs, six chains, temperature set to 0.2, sampling every 100th generation) until convergence parameters were met after 1.27 M generations, standard deviation having fallen below 0.01. Finally, a full search for the best-scoring maximum likelihood (ML) tree was performed in RAxML v.8.2.12 (Stamatakis 2014) using the standard search algorithm (same partitions, GTRCAT model, 2000 bootstrap replications). The significance threshold was set above 0.95 for posterior probability (PP) and 70% bootstrap proportions (BP).

The second alignment included only ITS sequences of *Leprocaulaceae* and *Catillariaceae* (using *Catillaria lenticularis* as outgroup because of its external position outside *Catillaria* s. str.). The purpose of this analysis was to resolve the different species in *Catillaria* s. str. and compare their intraspecific variability with that observed in other species of these families. Sequences (Table 1) came mainly from the same sources. The sequences were first aligned in MEGA 5.0 and then subjected to GBlocks (Castresana 2000) to remove 345 ambiguously aligned sites, resulting in a new dataset with 204/458 variable sites among 44 sequences. This alignment was loaded in MrBayes v.3.2.6, where a Bayesian analysis was performed (data not partitioned, GTR + G + I model, two simultaneous runs, four chains, temperature set to 0.2, sampling every 100th generation) until convergence parameters were met after 0.2 M generations, standard deviation having fallen below 0.01. A full search for the best-scoring ML tree was performed in RAxML v.8.2.12 using the standard search algorithm (data not partitioned, GTRGAMMA model, 2000 bootstrap replications). The significance threshold was set again at 0.95 for posterior probability (PP) and 70% bootstrap proportions (BP).

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Table 2. Comparison of the main characters of the six known corticolous *Catillaria* s. str. species. Information obtained from Kilias (1981), Tretiach & Hafellner (1998), van den Boom & Etayo (2001), Smith *et al.* (2009), van den Boom (2010) and the present study.

Species	Thallus	Apothecia (mm)	Margin	Disc	Ascospores (µm)	Pycnidia (µm); conidia (µm)	Ecology
C. chalybeia	thin, smooth to somewhat scurfy, grey-brown to very pale brown, ±slightly shiny	0.2–0.5	thin, concolorous with the disc, rarely paler	black or bluish black	9.5–12.2 × 3.1–4.1; 8 per ascus	50–80(–100); 1.8–3.5 × 0.5–0.8(–1.2)	saixcolous or corticolous only on *Fraxinus* trees
C. flexuosa	thick, uneven, knobby, ±subsquamulose, dark green, with ±pale brown tinge, matt	0.3–0.9	thick, often flexuose, concolorous with the disc	black	(6–)7–10(–11) × 2.5–3.5(–4); 8 per ascus	100–150; 2–3 × 0.9–1.2	corticolous only on trunks of *Fraxinus* trees
C. fungoides	very thin, smooth, whitish to pale grey, sorediate, shiny	0.2–0.4	thin, concolorous or slightly paler than the disc, finally excluded	black	10–12 × 3–3.5; 8 per ascus	unknown	corticolous on a wide range of phorophytes
C. nigroclavata	very thin, smooth, greyish, shiny	0.1–0.3	thin, concolorous or slightly paler than the disc	dark brown to black	8–10 × (2–)2.5(–4); 8 per ascus	unknown	corticolous on a wide range of phorophytes
C. praedicta	smooth to rimose-areolate, pale brown to dark brown or olivaceous, matt	0.25–0.5(–0.8)	thick, concolorous with the disc	brown to almost black	(4.5–)5.5–7.2(–9) × (2–)2.2–3(–3.5); 24–32 per ascus	100–150; 2.5–3 × 1.5	corticolous or lignicolous, on a wide range of phorophytes
C. subpraedicta	scurfy to farinose spongy granular; pale to dark brown, matt	0.2–0.4(–0.5)	thin, somewhat paler than the disc	dark grey to blackish	6.8–7.9 × 2.7–2.8; 16 per ascus	c. 75; 2.6–2.8 × 1.1	corticolous on a wide range of phorophytes

Results and Discussion

According to the present phylogenetic studies (Figs 1 & 2), the specimens studied are closely related to *Catillaria chalybeia*, the type species of *Catillaria*, represented by sequences obtained from saxicolous (*R. Haugan* 7947/ O-L-155291) and corticolous (*P. v. d. Boom* 59147, 59646) samples. A saxicolous collection found on the Azores Islands (*P. v. d. Boom* 55102) seems to be unrelated to the other lineages, being *c.* 60% similar in ITS, (475/782 bp identical), or 90% if a large 250 bp insertion in the ITS1 region is excluded, and is provisionally identified here as *Catillaria* sp. A third clade is composed of sequences of two collections with flexuose apothecia and differences of only 1.5% between them (11/784 bp), but both being *c.* 91% similar to sequences of *C. chalybeia* (712/781 bp). Regarding LSU, the three lineages differ in *c.* 2–3% (10–20/645 bp different), with a very low intraspecific variability (2/645 bp variable sites in both *C. chalybeia* and the clade with flexuose apothecia). In turn, all these samples are related to specimens identified as *C. nigroclavata* (G.M. 2015-07-09.5 (LUX), EDNA09-02107 (E) and EDNA09-02108 (E)), although the ITS sequence of EDNA09-02108 (E) seems to differ in up to 4.5% (21/477 bp) from the other two, maybe indicating that they come from different taxa. While the exact identity of *C. chalybeia* needs to be further confirmed by checking additional collections and appointing an epitype, the specimens with flexuose apothecia clearly represent a distinct taxon, a new name for which is proposed here.

All these species form a significantly supported clade that is considered here to be the core of *Catillaria*. Unfortunately, the genetic limits between *Catillaria* and the closely related genera *Halecania*, *Leprocaulon*, *Solenopsora* and *Speerschneidera* are not clear enough, probably due to the incomplete genetic data from most collections analyzed (Table 1). Synonymies between some of these genera cannot be rejected and, in this case, *Catillaria* (A. Massal. 1852) should be the priority name. In the present analysis, *C. lenticularis* seems to be unrelated to the core of the genus *Catillaria*. The limits between the families *Catillariaceae* and *Leprocaulaceae* are not clear either, maybe also being synonyms, in which case *Catillariaceae* (Hafellner 1984) should be given priority.

Taxonomy

Catillaria flexuosa van den Boom & P. Alvarado sp. nov.

MycoBank No.: MB 835063

Distinguished from *Catillaria chalybeia* by the dark green, relatively thick thallus, up to 0.4 mm, continuous to weakly rimose, areolate, with knobby granules, sometimes becoming subsquamulose. Apothecia 0.3–0.9 mm diam., often flexuose; smaller ascospores, (6–)7–10(–11) × 2.5–3.5(–4) µm, larger pycnidia 100–150 µm diam., and differing conidia, ellipsoid, 2–3 × 0.9–1.2 µm.

Type: The Netherlands, Noord-Brabant Prov., S of Veghel, S of Zijtaart, *Fraxinus* trees (medium size) along road, grid ref. 45-56-51, 51°35.3′N, 5°32.5′E, 10 m, 12 August 2019, *P. van den Boom* 58587 (B—holotype; hb. van den Boom—isotype). GenBank Accession nos: MT248986 (ITS rDNA), MT248984 (28S rDNA).

(Fig. 3A–C)

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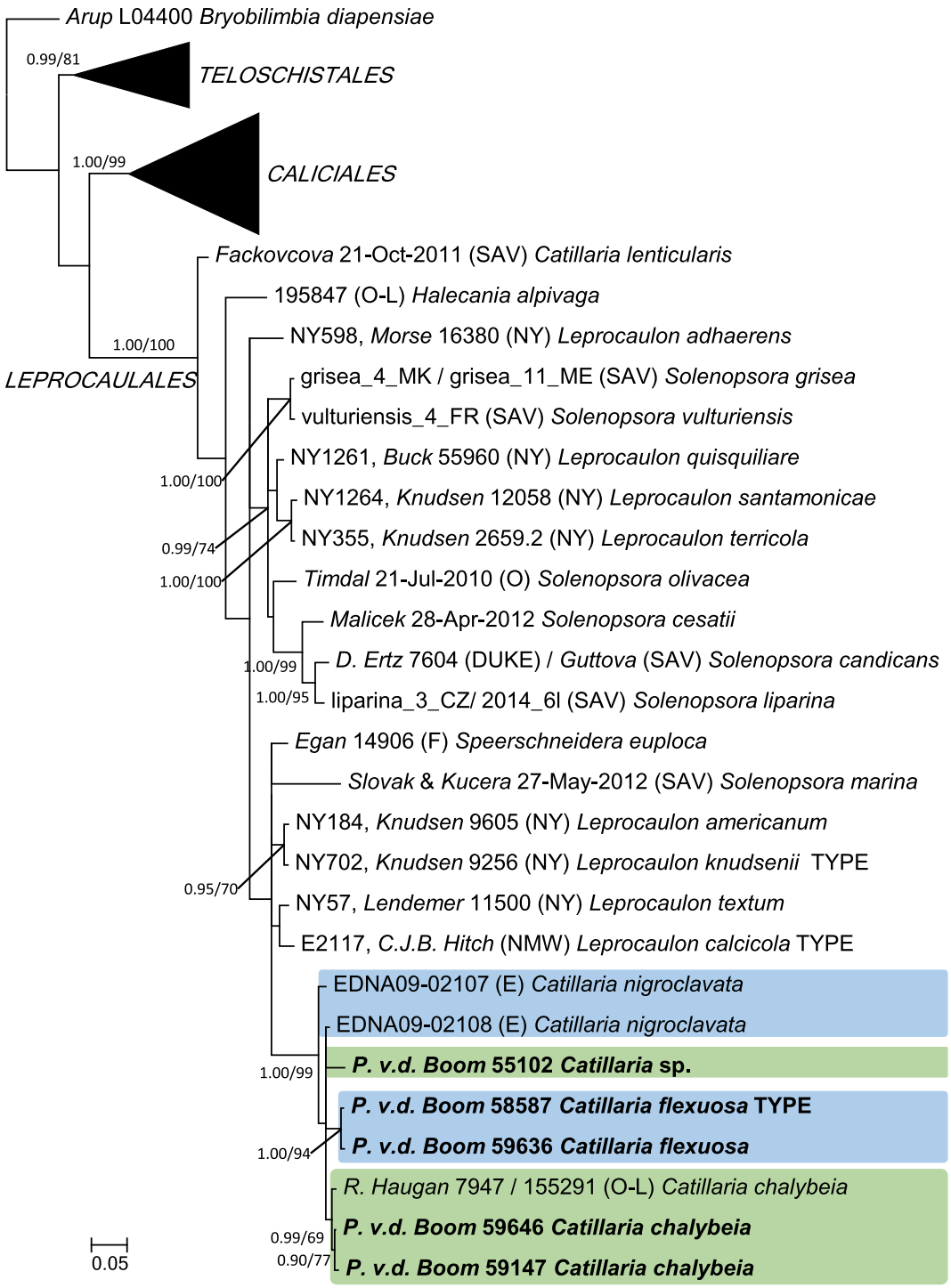

Fig. 1. A 50% majority rule ITS rDNA-28S rDNA-mtSSU-*Mcm7*-*tub2* consensus phylogram of the family *Catillariaceae* (with a selection of species from the orders *Teloschistales* and *Caliciales*, as well as *Bryobilimbia diapensiae* as outgroup) obtained in MrBayes from 23 925 sampled trees. Nodes were annotated if supported by ≥ 0.95 Bayesian posterior probability (PP) (left) or ≥ 70% maximum likelihood bootstrap proportions (BP) (right). Sequences newly generated in this study are in bold. In colour online.

Thallus relatively thick, up to 0.4 mm, continuous to weakly rimose, areolate, with knobby granules, sometimes becoming subsquamulose, growing in patches up to 1 cm wide, dark green, often with a brownish tinge, matt; *prothallus* not present; *photobiont* chlorophycean, cells 5–15 µm diam.

Apothecia abundantly present, 0.3–0.9 mm diam., flat, rarely slightly convex; *proper margin* conspicuous, presenting as a small rim especially in young apothecia, often flexuose, especially in mature apothecia (30–60 µm wide), persistent, somewhat shiny; *disc* black, matt; *true exciple* dark brown to blackish, sometimes with greenish pigments, mainly in young apothecia; *hymenium* 40–50 µm high, not presenting oil droplets; *epithecium* dark brown to greenish black, mainly in young apothecia, without crystals, K–; *hypothecium* dark

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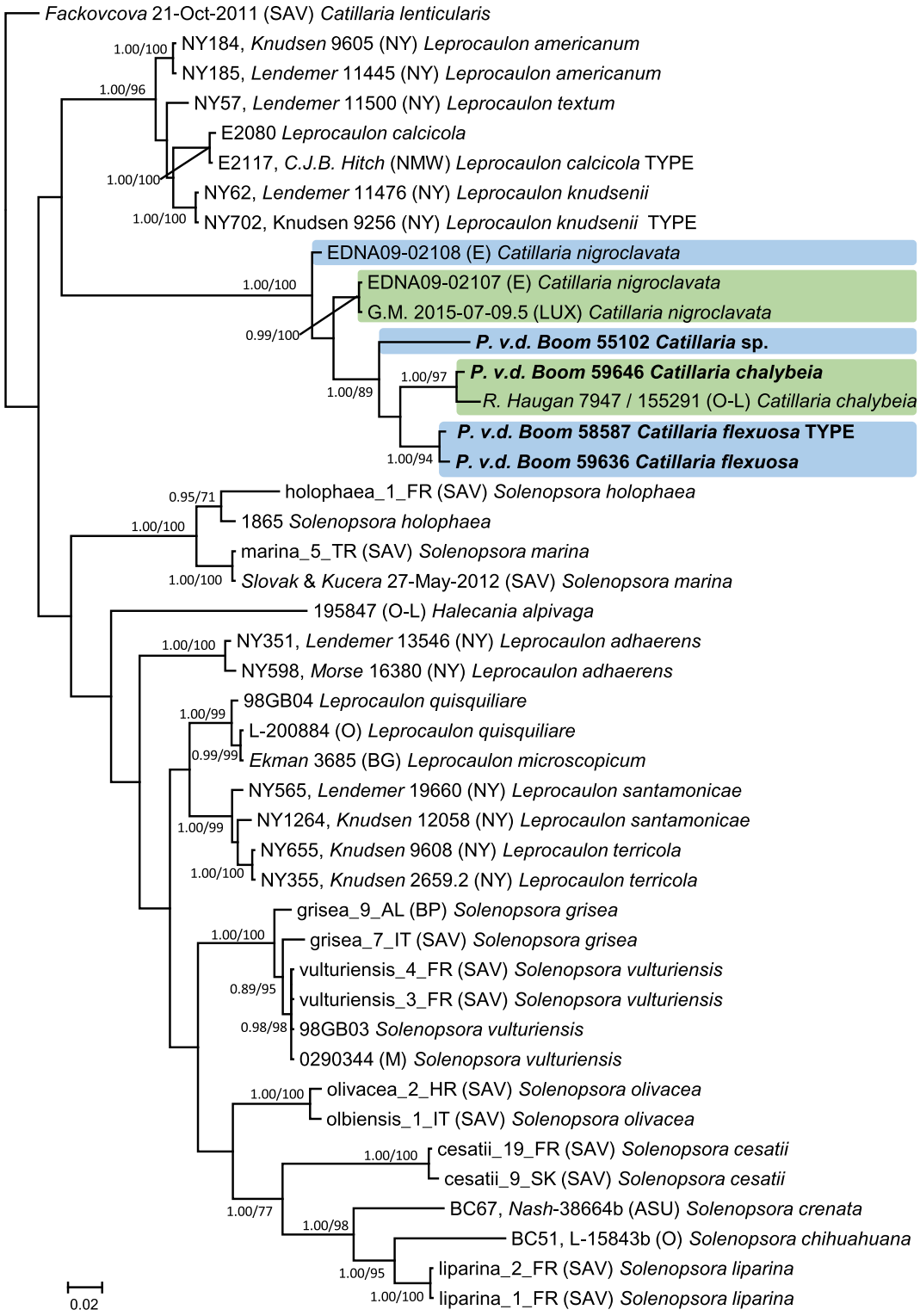

Fig. 2. A 50% majority rule ITS rDNA consensus phylogram of the family *Catillariaceae* (with a selection of species from *Leprocaulaceae*, as well as *Catillaria lenticularis* as outgroup) obtained in MrBayes from 1500 sampled trees. Nodes were annotated if supported by ≥ 0.95 Bayesian posterior probability (PP) (left) or ≥ 70% maximum likelihood bootstrap proportions (BP) (right). Sequences newly generated in this study are in bold. In colour online.

brown, K−; *paraphyses* simple to rarely sparingly branched, 1–1.5(–2) µm wide, septate, apices dark brown to blackish, widened, 3–6 µm diam.; *asci* cylindrical-clavate to clavate, 8-spored, *Catillaria*-type, with a uniformly blue apical dome in K/I, 35–40 × 8–12 µm; *ascospores* ellipsoid, (6–)7–10(–11) × 2.5–3.5(–4) µm, 1-septate, thin walled, often with oil droplets, not or only rarely slightly constricted at septum.

Pycnidia often present, immersed to somewhat erumpent, 100–150 µm diam., dark brown to blackish; *conidia* ellipsoid, hyaline, 2–3 × 0.9–1.2 µm.

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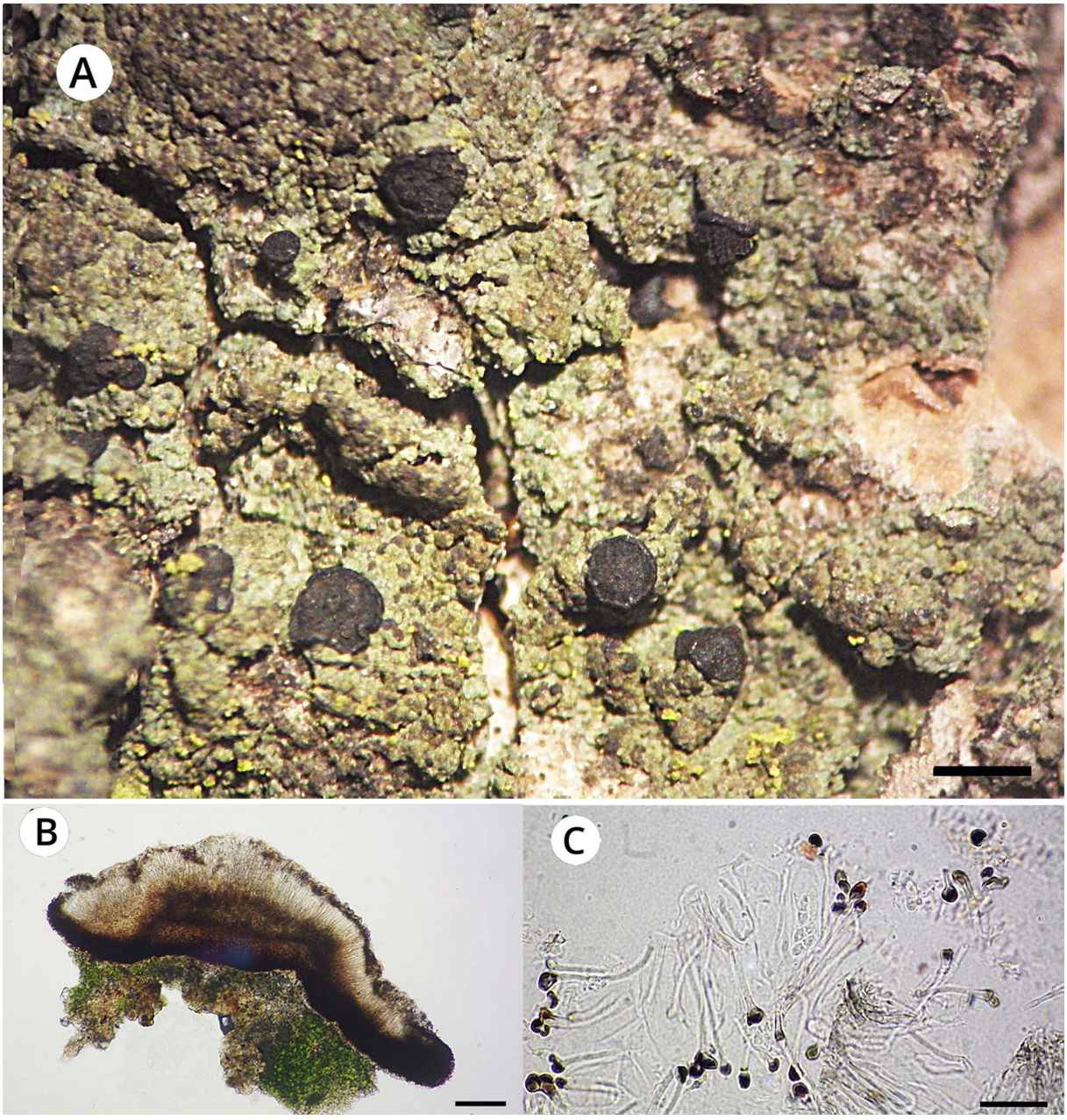

Fig. 3. *Catillaria flexuosa* (holotype, *P. van den Boom* 58587). A, habitus. B, section of apothecium. C, paraphyses and some ascospores. Scales: A = 1 mm; B = 100 µm; C = 20 µm. In colour online.

Chemistry. No chemical compounds detected by TLC. No crystals in thallus or apothecia.

Etymology. The epithet refers to the habitus of the apothecia.

Ecology and distribution. This new species is frequent in the type locality, growing on bark of wayside *Fraxinus excelsior* trees. It has a rather small distribution, being known from only five localities in the area, in the east of the province of Noord-Brabant. Other lichen species occurring at the type locality include *Candelaria concolor* (Dicks.) Stein, *Candelariella reflexa* (Nyl.) Lettau, *C. vitellina* (Hoffm.) Müll. Arg., *Catillaria nigroclavata* (Nyl.) Schuler, *Phaeophyscia nigricans* (Flörke) Moberg, *P. orbicularis* (Neck.) Moberg, *Physcia adscendens* H. Olivier, *P. caesia* (Hoffm.) Fürnr., *Physconia grisea* (Lam.) Poelt and *Xanthoria parietina* (L) Th. Fr. In addition, several lichenicolous fungi were found, including *Arthonia epiphyscia* Nyl. (very rare in the Netherlands), *A. parietinaria* Hafellner & A. Fleischhacker and *Lichenochora weillii* (Werner) Hafellner & R. Sant.

Notes. The morphological features of *Catillaria chalybeia* are described in detail by Kilias (1981), who chose the lectotype from a collection found by Borrer, on tiles and flints, housed in BM. The thallus is usually 80–200 µm thick, rimose-areolate to verrucose-areolate, areoles 0.1–0.4 mm wide, sometimes very poorly developed, beige to olive-brown, dark brown to black, often slightly shiny, and the prothallus is often not present or dark brown to black. Apothecia are usually 0.2–0.5 mm diam.,

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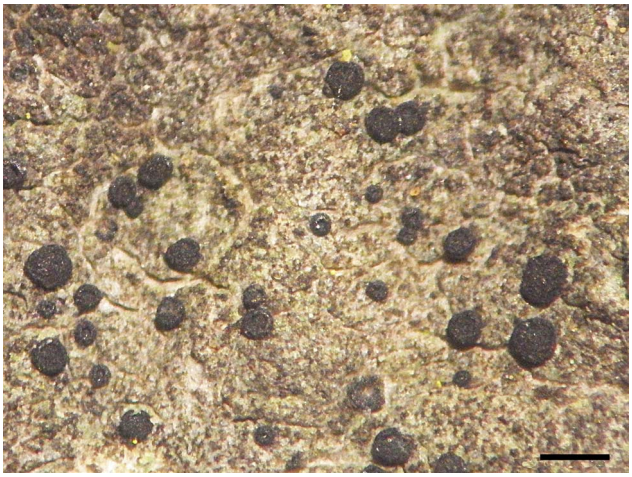

Fig. 4. *Catillaria chalybeia* (corticolous, *P. van den Boom* 59192), habitus. Scale = 0.5 mm. In colour online.

constricted at the base, the disc dark brown to black, excipulum black at outer rim, paler at inner side, asci 30–50 × 10–15 µm, ascospores 9.5–12.2 × 3.1–4.1 µm, pycnidia 50–80(–100) µm diam., and conidia bacilliform, 1.8–3.5 × 0.5–0.8 (–1.2) µm. Kilias (1981) listed many records from all over Europe.

Catillaria chalybeia is commonly found growing saxicolous in the Netherlands, Belgium and Luxemburg (Diederich *et al.* 2020), but in the present work it was also found corticolous (Fig. 4). Many saxicolous collections from the study area have been checked; they usually grew on brick and on schist, always appeared with a thin, smooth, rimose areolate, greyish to greyish brown slightly shiny thallus, and apothecia that are up to 0.5 mm diam., roundish, without a flexuose margin, often clustered, some slightly deformed. Corticolous specimens differ somewhat in thallus colour, with a more brownish tinge.

Catillaria flexuosa could be easily confused with *C. chalybeia* but differs by its matt, relatively thick greenish thallus, larger apothecia (up to 0.9 mm wide) often with a flexuous margin, smaller ascospores, and somewhat thicker conidia. In the field, *C. flexuosa* as well as the corticolous *C. chalybeia* form small and scattered inconspicuous patches, not exceeding 1 cm in width, and with no more than a few patches on isolated tree trunks.

Catillaria nigroclavata is also very common in the distribution area of the new species and both grow close to each other, and with *C. chalybeia*. *Catillaria nigroclavata* has much smaller apothecia, measuring *c.* 0.1–0.3 mm diam., and a very thin to immersed thallus with a greyish tinge. It is often found widely scattered on tree bark, but also on branches. *Catillaria fungoides* Etayo & van den Boom is also common in the studied area. Its apothecia resemble those of *C. flexuosa* but they have a thin, somewhat shiny thallus, and characteristic abundant black soralia; further differences to the aforementioned and to *Catillaria praedicta* Tretiach & Hafellner and *C. subpraedicta* M. Brand & van den Boom are provided in Table 2. In the field, *C. flexuosa* can be easily confused with *Amandinea punctata* (Hoffm.) Coppins & Scheid., a species with quite similar apothecia but with a more conspicuous, greyish thallus and brown ascospores.

Additional specimens examined. **The Netherlands:** *Noord-Brabant*: SSW of Erp, S of Keldonk, Rad to Diepers, *Fraxinus* trees (medium size) along road, grid ref. 51-16-14, 51°35′N, 5° 35.1′E, 10 m, 2019, *P.* & *B. van den Boom* 59120 (hb. v. d. Boom); W of St Oedenrode, S of road to Olland, road to Bobbenagelse Brug, corticolous on *Fraxinus* tree, grid ref. 51-14-34, 51°34.1′N, 5°25.8′E, 10 m, 2019, *P. van den Boom* 59211 (hb. v. d. Boom); N of Uden, De Maashorst, Menzel, Menzelsch Veld, Koudenoord, roadside *Fraxinus* trees along field, grid ref. 45-36-34, 51°41.9′N, 5°35′E, 15 m, 2020, *N. Ettema* & *P. van den Boom* 59636 (hb. v. d. Boom); Veghel, NE of city, W of road to Mariaheide, *Fraxinus* trees along small road, just outside housing estate, grid ref. 45-56-13, 51°37.55′N, 5°34.3′E, 10 m, 2020, *P.* & *B. van den Boom* 59644 (hb. v. d. Boom).

Catillaria chalybeia *(corticolous), specimens examined.* **The Netherlands:** *Noord-Brabant*: W of St Oedenrode, S of road to Olland, road to Bobbenagelse Brug, on *Fraxinus* tree, grid ref. 51-14-34, 51°34.1′N, 5°25.8′E, 10 m, 2019, *P. van den Boom* 59147 (hb. v. d. Boom); Breugel, Eind, near crossing to centre of village and road to Lieshout, roadside trees (*Tilia* and *Fraxinus*), on *Fraxinus*, grid ref. 51-25-35, 51°31.2′N, 5°31.4′E, 2019, *P.* & *B. van den Boom* 59192 (hb. v. d. Boom); Veghel, NE of city, W of road to Mariaheide, on *Fraxinus* trees along small road, just outside housing estate, grid ref. 45-56-13, 51°37.55′N, 5° 34.3′E, 10 m, 2020, *P. van den Boom* 59646 (hb. v. d. Boom).

Catillaria chalybeia *(saxicolous), selected specimens examined.* **The Netherlands:** *Noord-Brabant*: Eindhoven, NNW of Geldrop, road to Urkhoven, on brick of culvert, grid. ref. 51-46-31, 51° 26.0′N, 5°32.2′E, 20 m, 1993, *P.* & *B. van den Boom* 13805 (hb. v. d. Boom); Valkenswaard centre, churchyard since 1887, tombstones of concrete, brick, schist etc., on schist, grid. ref. 57-14-25, 51°21.1′N, 5°27.5′E, 20 m, 2009, *P.* & *B. van den Boom* 43474 (hb. v. d. Boom); Eindhoven centre, churchyard, gravestones of concrete and schist, on schist, grid. ref. 51-44-15, 51°27.1′N, 5°27.5′E, 20 m, 2014, *P.* & *B. van den Boom* 51920 (hb. v. d. Boom).

Catillaria *sp. (saxicolous), specimen examined.* **Portugal:** *Azores*: Faial, W side of island, NNE of Praia do Norte, Baia da Ribeira das Cabras, coastal area, some scattered trees, outcrops and walls with volcanic stones along field, on volcanic stone, 38°36.72′N, 28°45.74′W, 35 m, 2016, *P.* & *B. van den Boom* 55102 (hb. v. d. Boom).

Arthonia epiphyscia, *specimens examined.* **The Netherlands:** *Noord-Brabant*: S of Veghel, S of Zijtaart, *Fraxinus* trees (medium size) along road, grid ref. 45-56-51, 51°35.3′N, 5°32.5′E, 10 m, 2019, *P. van den Boom* 58591 (hb. v. d. Boom); N of Uden, De Maashorst, Menzel, Menzelsch Veld, Koudenoord, roadside *Fraxinus* trees along field, grid ref. 45-36-34, 51°41.9′N, 5°35′E, 15 m, 2020, *N. Ettema* & *P. van den Boom* 59640 (hb. v. d. Boom).

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