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March 25<sup>th</sup> - 29<sup>th</sup>, 2017

3<sup>rd</sup> International Conference on Avian herpetological  
and Exotic mammal medicine

**PROCEEDINGS**

ORGANIZED BY



European Association  
of Avian  
Veterinarians



Association  
of Exotic Mammal  
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Association of Reptilian  
and Amphibian  
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European  
College of  
Zoological Medicine

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**TUESDAY**  
**28<sup>th</sup> March 2017**

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**Scientific sessions**  
**herpetology**

Scientific session HERPETOLOGY - **Reproduction**

**ULTRASOUND SEX DETERMINATION IN SNAKES:  
CORRELATION BETWEEN HEMIPENES  
OR HEMICLITORIDS AND  
BODY MORPHOLOGICAL PARAMETERS**

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**ABSTRACT**

In practice, snakes' sex is mostly determined using invasive techniques such as probing, popping or endoscopy. Ultrasonography has been used as a high sensitivity and non invasive technique in snake's sex determination since 2009, but few studies are still available and none of them ever considered the dimensions of copulatory organs or tried to correlate them to the morphometric parameters of snakes.

From August to October 2016, fifty snakes of the *Pythonidae*, *Boidae* and *Colubridae* families underwent the ultrasonographic examination of hemipenes and hemiclitoris in order to confirm their sex. Hemipenes and hemiclitoris (right and left) length, height and width were ultrasonographically measured in longitudinal and transverse scans of the cloacal region. At the same time, the body length, the tail length, the head length and width and the diameter of the cloacal segment of each snake was measured and recorded on a worksheet. The procedures never required anesthesia or sedation of the snakes, which were only manually restrained.

We confirm that ultrasonography is a sensitive, safe and harmless technique for snakes' sex determination. In addition, we found statistically significant correlations between hemipenes and morphometric parameters, but no correlation was found involving the hemiclitoris.

**1. INTRODUCTION**

As snakes do not show any fair sexual dimorphism (except for few species), the application of sex determination techniques is required.

Even though small physical variations exist between males and females, like size difference in big constrictors<sup>1</sup> or size of the cloacal spurs,<sup>2</sup> these are only statistic data, not applicable to an individual.

A very common technique between *Python regius* breeders is the evaluation of the length of the tail, which is longer in males. This method has no scientific fundament.<sup>3</sup>

Another well known technique is the use of an atraumatic probe, inserted the in the invaginated hemipenis or hemiclitoris. It's a simple repeatable and cheap method,<sup>2</sup> but the risk of injuring the copulatory organ and perforate the hemiclitoris and the tail,<sup>4</sup> or to vehicle serious infections with severe consequences is described.

On the opposite, the probe could be inserted partially, due to the presence of dense secretion and give wrong results.<sup>2</sup>

A widely used technique (especially for juvenile Colubridae) is the manual eversion of hemipenis. With finger pressure, the operator produces the prolapse of the organs, making them explorable. The disadvantage is the probability of traumatism and fractures of the spine and the ribs. It is also possible to fail seeing the hemipenis.<sup>2</sup>

The "saline injection", makes the hemipenis pro-

lapse, using the hydrostatic pressure of a sterile saline solution injected in the tail of the snake.

This gives a better vision of hemipenis and hemiclitoris. Unfortunately this method has also its troubles: injecting the liquid into the hemipenis could cause severe injuries.

Both eversion techniques are not applicable to large snakes, due to the opposition of the hemipenis retractor muscle.<sup>2</sup>

The use of a cystoscope instead of the probe gives also good results in clinical practice; however, they have the same invasiveness.

Endoscopy has been used to visualize the gonads, after a surgical preparation of a coelomatic access. It is a procedure with all the risks of a surgical anesthesia and with high cost due to the instrumentation.<sup>2</sup>

Ultrasonography has been considered a good method to observe the reproductive system of snakes as it helps to identify gonads modifications during the reproductive cycle.

In females the ovary could be detected dorsally or laterally to the kidney (due to the mesovary laxity) and the follicles can be easily seen in their maturation stages.<sup>2</sup>

Oviduct can also be seen in every phase of the reproductive cycle.

In males, testes can be identified cranially to the kidneys.<sup>5</sup>

The ultrasonographic appearance of hemipenis has been described in literature.<sup>5,6</sup>

## 2. MATERIALS AND METHODS

For this study, we used a portable ultrasound machine Esaote® MyLab Five, equipped with a linear multifrequency 8-12 MHz probe.

All the snakes examined were propriety of the reptilium section of "Zoo Safari Park", Pombia (NO), of the wildlife park "Le Cornelle", Valbrembo (BG), of "Oasi di Sant'Alessio", Sant'Alessio (PV) and of the Natural Science Gallery and herpetological Centre "Reptiland", Riva del Garda (TN).

### 2.1 Observational study

This study comprehended snakes belonging only to *Boidae*, *Pythonidae* and *Colubridae* families. Animals with evident disease were excluded by this study.

Every specimen has been taken from its terrarium and physically restrained by a keeper and/or a

proficient veterinary, in order to limit stress. No pharmacological restrain has been used.

### 2.2 Measurement

Keeping the animal in dorsal decubitus, an operator applied ultrasound gel on the analyzed body part and took three scans.

In the longitudinal scans of hemipenis/hemiclitoris, right and left, the echotexture of the copulatory organ was detected and digitally recorded. The maximum measurable length and height of each hemipenis/hemiclitoris were considered in these scans.

In the transverse section, scanning from the cloacal scale towards the tail, the maximum measurable height and width of each hemipenes/hemiclitoris were obtained.

Basing on the echographic appearance, the gender of the snake was determined.

After the ultrasonographic examination, the following morphometric measures were taken using a tape measure and a digital gauge.

- Length of the snake (from the nose to the end of the tail)
- Distance between the cloaca and the tip of the tail
- Pelvis diameter at the cloaca
- Length of the head (from the caudal end of the square bones to the snout)
- Width of the head: (from one parietal bone to another).

All the data were filed in an Excel® table and later classified for family, genus and species.

For each of these groups, average and standard deviation of morphometric and echographic measurements were calculated. For each subject, average and standard deviation between the measurements of the right and the left hemipenes/hemiclitoris were considered.

### 2.3 Statistical analysis

The presence of a statistically significant correlation between the morphometric measures and the averages of the measures of the hemipenes/hemiclitoris was determined (MedCalc®). Only groups of at least 3 specimens were considered.

The statistical significance was considered for  $p < 0,05$ , with confidence interval at 95%, while the level of confidence was defined by these criteria: weak for  $r < 0,3$ ; mild for  $0,3 < r < 0,7$ ; strong for  $r > 0,7$ .

The t-test for independent values was performed to assess the existence of a significant difference ( $p < 0,05$ ) between the morphometric values of the two genders.

**3. RESULTS**

The studied sample consisted in 55 snakes: 5 *Boidae*, 25 *Colubridae* e 25 *Pythonidae*.

*Boidae* comprehended 3 *Boa constrictor* (2 females and 1 male), 1 *Eunectes notaeus* (female) and 1 *Acrantophis dumerili* (male).

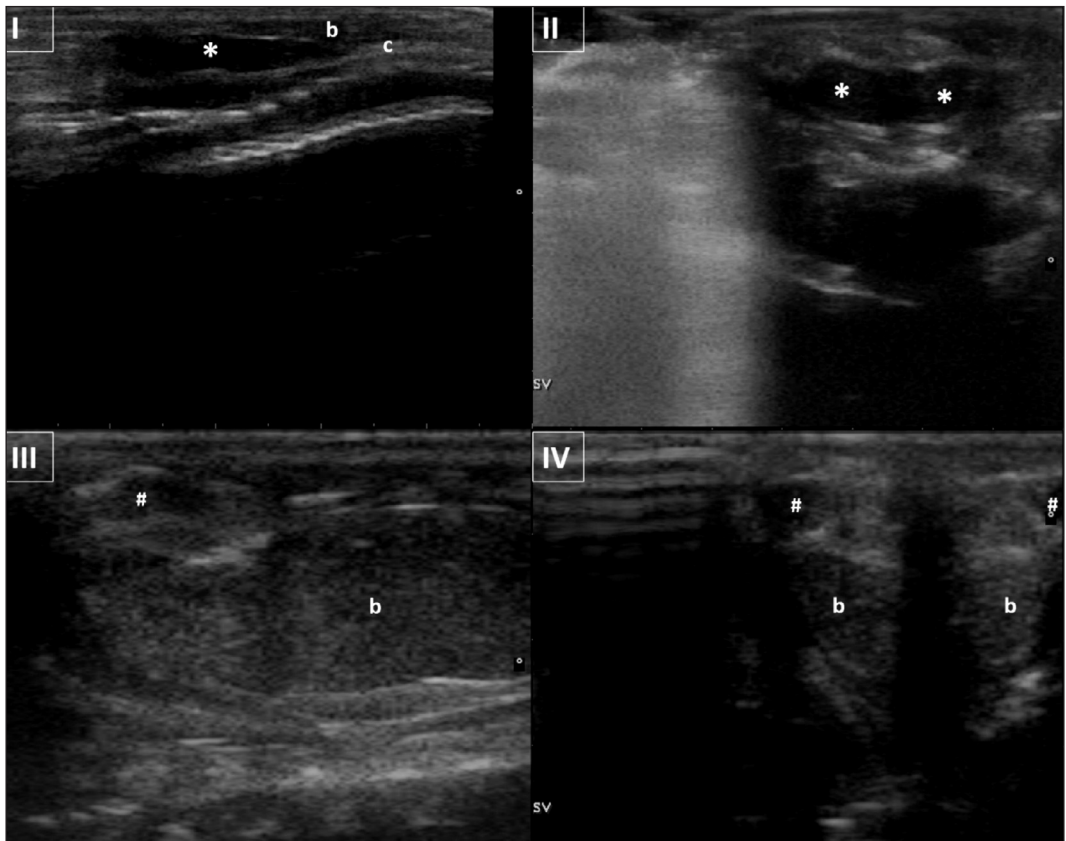
*Colubridae* comprehended 3 *Lampropeltis alterna* (2 males, 1 female), 3 *L. getula* (2 males, 1 female), 1 *L. pyromelana* (male), 7 *L. triangulum* (2 males, 5 females), *Pantherophis guttatus* (7

males, 1 female), 2 *Orthriophis taeniurus* (1 male e 1 female), *Pituophis melanoleucus* (male).

*Pythonidae* comprehend 1 *Liasis olivaceus* (female), 1 *Morelia spilota* (female), 1 *M. viridis* (male), 2 *Python curtus* (males), 7 *P. bivittatus* (4 males, 3 females), 12 *P. regius* (7 males, 5 females), 1 *P. reticulatus* (female).

The hemipenes (Fig. 1, A) showed an anechoic or hypoechoic structure, characterized by small echogenic foci and a hyperechoic and defined border. They were triangular shaped with the base near the cloaca (longitudinal scan) and a circular section (transversal scan). It was possible to find their opening near the cloaca.

Caudally it was possible to see the retractor mus-



**Figure 1** - I: longitudinal scan of a male *Acanthophis dumerili*, showing the triangular shaped markedly hypoechoic hemipenis, portion of the hypoechoic scent gland, and the hyperechoic retractor muscle; II: transverse scan of a male *Acanthophis dumerili*, showing the two hemipenes; III: longitudinal scan of a female *Python regius*, showing the irregularly shaped hypoechoic hemiclitoris and the heterogeneous scent gland; IV: transverse scan of a female *Python regius*, showing the two hypoechoic hemiclitoris and the scent glands. \*: hemipenis, b: scent gland, c: hemipenis retractor muscle, #: hemiclitoris.

TUESDAY 28<sup>TH</sup> MARCH  
Herpetology

cle (Fig. 1, c), more echoic and homogeneous. The scent gland, in males, was more echoic and homogeneous. It was dorsal to the hemipenis near the cloaca and becoming ventral near the tail. Hemiclitoris were more difficult to find, always smaller than the hemipenes and sometimes asymmetrical. Their shape was irregularly ovoid, they looked mostly hypoechoic, with great variations, depending on their contents. They were always cranial to the scent gland that occupied the most of the tail, caudal to the cloaca.

In *Colubridae* family, the average length of the hemipenes was  $2,32 \pm 0,7$  cm, while for the hemiclitoris was  $0,67 \pm 0,26$  cm. The height, measured in longitudinal scan, was  $0,41 \pm 0,13$  cm for the hemipenes, and was  $0,18 \pm 0,04$  cm for the hemiclitoris. The height, measured in transversal scan was respectively  $0,39 \pm 0,13$  cm and  $0,18 \pm 0,03$  cm. The average width of the hemipenis was  $0,45 \pm 0,12$  cm and for the hemiclitoris was  $0,23 \pm 0,05$  cm.

The length of the snake, the diameter of the pelvis and the width of the head were mildly correlated to all the echographic measurements of the hemipenis.

In *Boidae* family, the average length of the hemipenes was  $2 \pm 0,16$  cm, while the average length of the hemiclitoris was  $0,81 \pm 0,3$  cm; the heights were  $0,43 \pm 0,05$  cm for males and  $0,18 \pm 0,07$  cm for females. In transverse scans were respectively  $0,42 \pm 0,03$  cm and  $0,18 \pm 0,06$  cm. The width of the hemipenis was  $0,51 \pm 0,01$  cm and the width of the hemiclitoris was  $0,25 \pm 0,03$  cm. A strong correlation was found only between the length of the head of the females and the average height of the hemiclitoris (measured in transverse scan).

In the *Pythonidae* family, the average length of the hemipenes was  $2,55 \pm 1,25$  cm, while the average length of the hemiclitoris was  $1,03 \pm 0,37$  cm. The height measured in longitudinal scan was  $0,51 \pm 0,32$  cm for the hemipenes and  $0,19 \pm 0,04$  cm for the hemiclitoris. In transverse scan, the heights were respectively  $0,51 \pm 0,37$  cm and  $0,22 \pm 0,04$  cm; the width was  $0,58 \pm 0,32$  cm for the hemipenis and  $0,31 \pm 0,05$  cm for the hemiclitoris.

Females of this family showed a mild correlation between the diameter of the pelvis and the width of the hemiclitoris and between the length of the head and the width of the hemiclitoris. Males showed a strong correlation between the

length of the snake, the length of the tail, the diameter of the pelvis and the length of the head, with all the measures of the hemipenes.

The width of the head had a mild correlation with the height and the width of the hemipenis. Considering the genera, statistical analysis was performed only for *Python spp.*, *Lampropeltis spp.* and *Pantherophis spp.*

*Lampropeltis spp.* showed a strong correlation between the diameter of the pelvis and the average width of the hemipenis. The length and the width of the head had a strong correlation with the height and the width of the hemipenis.

In *Pantherophis spp.* there was a strong correlation between length of the snake, the diameter of the pelvis and the length of the hemipenis.

*Python spp.* males had strong correlations between length of the snake, length of the tail, diameter of the pelvis, length of the head and all the parameters of the hemipenes.

The width of the head shows medium level of correlations with all the parameters of the hemipenes.

In females a strong correlation was found between all the morphometric measures and the length of the hemiclitoris.

Concerning the species, we observed that *P. bivittatus* had correlations comparable with those of the *Python* genus, while *P. regius* didn't.

#### 4. DISCUSSION

Use of ultrasound for gender determination in snakes proved to be a fast procedure and was well tolerated by the snakes: none of them showed signs of stress or pain, during the examination. Identification of the copulatory organs was easy and the post processing evaluation of the images avoided mistakes during the gender determination. We were able to obtain diagnostic images for all subjects, starting from 1 cm of pelvic diameter.

In big snakes, we found artifacts that limited the quality of the images, because of the thickness of the scutae. Eventually in only 3 subjects we were not able to measure at least one parameter, but it was always possible to find the copulatory organs in at least one scan (longitudinal or transverse).

The effectiveness of the ultrasound in sex determination of snakes, in our population was witnessed by a sensibility and specificity of 100%, compared to the usual probe techniques.

We consider that the substantial lack of correla-

tions in females, could be a consequence of the small size of the hemiclitoris, which produced a great data dispersion.

Considering the families *Colubridae* and *Pythonidae*, we observed that the size of the hemipenis was proportional to the size of the snake.

In the *Colubridae* we found an inferior number of correlations compared with the *Pythonidae*. We speculate that it was caused by the greater heterogeneity of *Colubridae*.

Even though there were few correlations found in *Boidae*, the exiguous number of subjects examined, didn't allow to consider them reliable. These data should be confirmed on a wider number of animals.

The absence of a significant difference of morphological parameters between males and females confirm that visual sex determination is unreliable. The great heterogeneity of our population resulted in interesting correlations mainly about the families. For the genera and for the species the fragmentation in different groups permitted to obtain correlations only for genera *Python*, *Lampropeltis*, *Pantherophis* and the species *P. bivittatus*.

The strongest correlations were those observed between: hemipenes and the length of the snake and hemipenes and diameter of the pelvis, observed in all the families, in almost all of the genera and in *P. bivittatus*.

For these correlations, we also calculated the ratios:

The ratio between the length of the snake and the length of the hemipenis was  $58,6 \pm 13,8$  for *Colubridae*,  $61,9 \pm 13,5$  for *Pythonidae*,  $59,8 \pm 11,7$  for *Python* and  $65,3 \pm 4$  for *P. bivittatus*.

The ratio between the length of the snake and the height of the hemipenis (longitudinal scan) was  $337,9 \pm 102$  for *Colubridae*;  $320,9 \pm 73,5$  for *Pythonidae*,  $304,8 \pm 43,8$  for *Python spp.* and  $296,7 \pm 19,2$  for *P. bivittatus*.

The ratio between the length of the snake and the height of the hemipenis (transverse scan) was  $353,5 \pm 114,9$  for *Colubridae*;  $328,3 \pm 66,3$  for *Pythonidae*,  $316,4 \pm 50,8$  for *Python spp.* and  $306,3 \pm 47,5$  for *P. bivittatus*.

The ratio between the length of the snake and the width of the hemipenis was  $294,5 \pm 73,2$  for *Colubridae*;  $277,6 \pm 50,2$  for *Pythonidae*,  $270,1 \pm 43,3$  for *Python spp.* and  $300,3 \pm 23,7$  for *P. bivittatus*.

The ratio between the diameter of the pelvis and

the length of the hemipenis was  $0,6 \pm 0,1$  for *Colubridae*,  $1 \pm 0,3$  for *Pythonidae*,  $1 \pm 0,3$  for *Python spp.* and  $1 \pm 0,2$  for *P. bivittatus*.

The ratio between the diameter of the pelvis and the height of the hemipenis (longitudinal scan) was  $3,5 \pm 1$  for *Colubridae*,  $5,3 \pm 1,3$  for *Pythonidae*,  $5,3 \pm 1,3$  for *Python spp.* and  $4,3 \pm 0,4$  for *P. bivittatus*.

The ratio between the diameter of the pelvis and the the height of the hemipenis (transverse scan) was  $3,7 \pm 1,2$  for *Colubridae*,  $5,4 \pm 1$  for *Pythonidae*,  $5,5 \pm 1$  for *Python spp.* and  $4,5 \pm 0,7$  for *P. bivittatus*.

The ratio between the diameter of the pelvis and the width of the hemipenis was  $3 \pm 0,7$  for *Colubridae*;  $4,5 \pm 0,6$  for *Pythonidae*,  $4,6 \pm 0,6$  for *Python spp.* and  $4,4 \pm 0,5$  for *P. bivittatus*.

In our experience ultrasonography confirmed to be an easy feasible, reliable, safe and harmless technique. It should be considered as an elective technique for sex determination in snakes. We think that our promising results could be considered for future studies aimed to establish reference values applicable to more genera and species.

*The permission for publication has been obtained from all the authors on this manuscript.*

*The study was performed in accordance with the ethical standard dictated by the applicable law.*

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## AUTHOR INDEX

### A

Abad C., 594, 740, 800  
 Abadie Jérôme, 731  
 Abou-Zahr Tariq, 400, 686  
 Abreu Sara, 800  
 Abu Jalila, 518  
 Agnetti Francesco, 398  
 Ahmad Abadi Hassan Nili, 665  
 Ahmad Madani Seyed, 667, 669, 671  
 Alessandria Valentina, 774  
 Alhgrim Kris, 369  
 Almeida Cristina, 407  
 Alves Teixeira Fábio, 448  
 Ammersbach Melanie, 360  
 Ancy George, 351  
 André M.R., 771  
 Anglister Nili, 480  
 Anleu Rony García, 476  
 Anselmi Carlo, 766  
 Antinoff Natalie, 307  
 Antonelli Maria Virginia, 766  
 Antonissen G., 350, 357, 511, 768  
 Arabkhazaeli Fatemeh, 671  
 Arbanasić H., 820  
 Arca-Ruibal Bárbara, 351  
 Arcangeli Andrea, 759  
 Ardiaca Garcia Maria, 357, 359, 416, 442, 444  
 Arnold Susan, 470  
 Asti Martina, 759  
 Attili Anna Rita, 790  
 Aupperle H., 809  
 Aupperle Heike, 582  
 Avni-Magen Nili, 571  
 Axelson Rick, 687  
 Aziz Saleha A., 518  
 Azmanis Panayiotis (Panos), 207, 673

### B

Bagherighadikolaei Seyed sina, 693  
 Balogh Marton, 563  
 Bandsma J. (Joost), 616  
 Bangoura B., 673  
 Barazorda Romero Silvia, 583, 588, 707  
 Barbero Rodríguez Sergio, 442  
 Barbero S., 359, 444  
 Barbero Sergio, 416  
 Bardi Edoardo, 404, 574, 672  
 Baron Hamish, 681  
 Barszcz Karolina, 617, 813  
 Bartels T., 473  
 Bartha Andrés, 806  
 Bartoskova Alena, 543  
 Barzegarinaeini Reza, 693  
 Baumgartner C., 709  
 Baur M., 384, 473  
 Beaufrere Hugues, 360, 571, 687, 690, 743  
 Bedin M., 482

Behrend Ellen N., 580  
 Bel Lucia Victoria, 704  
 Belic Maja, 786  
 Benato L., 744  
 Berkowitz Asaf, 691  
 Berman Justine, 507  
 Bernard Fabrice, 634, 635  
 Bert Elena, 394, 528  
 Bertagnoli S., 666  
 Bezerra Mamede Rafael, 466  
 Biascochea Jose, 369  
 Bielli Mattia, 261, 404, 552, 814  
 Bilek A., 728  
 Billet Jean-Philippe, 584, 636, 720  
 Binanti Diana, 768, 773, 802, 803, 815  
 Bishop Rebecca, 371  
 Blahak S., 380, 384, 473  
 Blasco E., 639, 390  
 Böhmer Estella, 333, 624  
 Bolen G., 741  
 Bolton D., 529  
 Bonfilii Laura, 689  
 Bonvehí Nadeu Cristina, 359, 416, 442, 444  
 Bordelo J., 375  
 Borges-Nojosa D.M., 410, 788  
 Boston Clark Terry, 369, 371  
 Boswijk-Appelhof H.C. (Lenie), 437  
 Bottinelli M., 381  
 Boyes E., 529  
 Bradley Charles, 694  
 Braga R.R., 410, 788  
 Brainard Benjamin, 570  
 Brambilla Chiara, 439, 603  
 Brandão João, 364, 365, 395, 517, 590, 613  
 Bravo Rada F. David, 580  
 Brethaut E., 741  
 Briganti A., 349  
 Briola Chiara, 394  
 Brooks Marjory, 570  
 Bublat A., 509, 538  
 Bücking Bianca, 656  
 Bujanić M., 820  
 Bulliot Christophe, 427, 433, 633, 762  
 Burmeister Anne-Kathrin, 485  
 Burresti Veronica, 672  
 Buttin Rachel, 433, 634, 635  
 Buxton Nikki, 476

### C

Caekebeke N., 357  
 Calabuig Pascual, 780, 795  
 Calandra Diana, 623  
 Calvo Carrasco Daniel, 400, 408, 674, 686, 692, 711, 755  
 Campoy Luis, 737  
 Camus Melinda S., 575  
 Canadas Ana, 770  
 Canturri Albert, 754

- Capello Vittorio, 157, 175, 186, 286  
 Carsana Gabriele, 786  
 Castro Pedro, 794  
 Carter Holly, 369  
 Carvalho L.M., 403  
 Cattarossi D., 482  
 Cavaliere Carciofi Aulus, 448  
 Cavina D., 756  
 Ceccherelli R., 349  
 Cepelcha Vaclav, 723  
 Cermakova Eva, 542, 558, 583, 707, 723  
 Cernochova Hana, 210  
 Chapel J.M., 607, 807  
 Charpentier Marie, 452, 469  
 Chassang Lucile, 367, 368, 417, 736  
 Chen Yu-Wei, 630  
 Chetboul Valérie, 565  
 Chi Chau Hwa, 713  
 Childress April L., 382  
 Chitty John, 30, 354, 529, 604  
 Chu Marty, 630  
 Ciembor Paula, 525  
 Cipone M., 607, 807  
 Ciprian Ober, 704  
 Ciraci Ivano Antonio, 394  
 Cizelj Ivan, 721, 791  
 Cocolin Luca, 774  
 Codea Razvan, 704  
 Coletti M., 792  
 Collarile Tommaso, 508  
 Colon Violaine, 567  
 Comolli Jessica, 575  
 Cope I., 426  
 Coquelle Mélanie, 731  
 Corbera Juan A., 780  
 Correa Kuci Cristiana, 777  
 Corso Altamir, 777  
 Corti C., 792  
 Cousin H., 733  
 Couto C., 771  
 Cox Sherry, 708  
 Cramer K., 473  
 Craveiro Hélder, 770  
 Cristofani Pierluigi, 773  
 Crosse Patricia, 731  
 Crossland Nicholas, 382, 494  
 Crosta Lorenzo, 30, 43, 277, 663  
 Croubels S., 350, 357, 738, 768  
 Cuere J., 594, 740, 800, 804  
 Curros Moreno Angel, 418, 753  
 Cusack Lara, 575, 579, 589  
 Cuteri Vincenzo, 790  
 Cutler Daniel, 575, 589  
 Czubaj Norbert, 617, 813
- D**
- d'Ovidio Dario, 751, 773  
 D'Ovidio Miriam, 420  
 Dahlhausen Robert D., 84  
 Dahlhausen Robert, 663  
 Damoiseaux Cécile, 565  
 Dawson Charlotte, 569  
 Dawson R., 477  
 Dayen M., 473  
 De Araujo Jeann Leal, 507  
 De Baere S., 350, 738, 768  
 De Besi P., 725  
 De Boer E.J. (Evert-Jan), 437  
 De Jaureguizar Texas Maria Rosa, 692  
 De la Fuente Gabriel, 443  
 De Luca C., 390, 482, 486  
 De Matos Ricardo, 737  
 De Moraes Aury Nunes, 777  
 De Oliveira Juliana Paula, 448  
 De Santi M., 771  
 De Souza Theodoro Stephanie, 448  
 Defarges Alice, 743  
 Deflers H., 741  
 Degorce-Rubiales F., 745  
 Del Piero Fabio, 386  
 Del Río Alonso Laura, 776, 782  
 Del Rossi E., 792  
 Deli Gianluca, 574, 577, 725, 759, 792, 804  
 Delverdier Maxence, 810  
 Demangeot Sophie, 636  
 Déniz Soraya, 794  
 Desmarchelier Marion, 457  
 Desprez Isabelle L., 580  
 Deutz Armin, 475  
 Devaux Lauriane, 731  
 Devreese M., 350, 357, 738, 768  
 Dhondt L., 768  
 Di Girolamo Nicola, 112, 418, 441, 445, 508, 621, 623  
 Di Giuseppe Marco, 543, 544, 572  
 Di Graci Salvatore, 404  
 Di Ianni Francesco, 412, 430  
 Di Mattia Diana, 429  
 Di Somma A., 673  
 Diana A., 607, 807  
 Dias I., 366, 375  
 Dias Sara C., 729, 754  
 Dieterly A.M., 395  
 Dietz Janosch, 582  
 DiGeronimo Peter M., 382, 494  
 Dini Fabrizio, 689  
 Divers Stephen J., 90, 98, 125, 316, 570, 575, 579, 589, 595, 688  
 Do Couto Caroline, 453, 466  
 Dogliero Andrea, 512, 774  
 Dolka Izabella, 776  
 Donnelly Thomas M., 180, 220  
 Dorresteijn Gerry, 476, 689, 781  
 Doss Grayson A., 372, 496, 621, 703  
 Drasch Katrin, 485  
 Dresco Thomas, 636  
 Driggers Todd, 369  
 Drijver E.M. (Evianna), 616  
 Dugat Danielle, 517  
 Duhamelle Alexis, 348, 637  
 Duncan Neil, 682

## E

Echenberg Alex, 371  
 Echols M. Scott, 80, 295, 325, 369, 371  
 Ehling C., 538  
 Eleuteri Annamaria, 689  
 Encinas T., 594, 740  
 Enderlein Dirk, 656, 658  
 Engelke Elisabeth, 555  
 Eördögh R., 728  
 Ermáková E., 94, 143  
 Eshar David, 571, 606  
 Espada Yvonne, 557  
 Eszter Kertész, 790  
 Evans Dawn E., 494  
 Evans Lauren, 613  
 Everett S., 529

## F

Fagulha Teresa, 770  
 Farnir F., 741  
 Fasolo Chiara, 434  
 Faulkner Emma, 590  
 Favaro da Cunha Anderson, 363, 710  
 Faverzani S., 547  
 Fazio Gabriella, 439, 603  
 Fehr Michael, 555, 614  
 Fernández Francisco, 729  
 Fernández Martín Elena, 805  
 Ferreira Xavier, 418, 461  
 Ferrell Shannon, 457  
 Ferrera Alvarez Ekei, 359, 416, 442, 444  
 Ferres I., 390  
 Ferrocino Ilario, 774  
 Ferrucci Francesco, 439, 603  
 Fevereiro Miguel, 770  
 Fichi Gianluca, 773  
 Fictum Petr, 781  
 Fink Dustin, 702, 703  
 Fischer D., 509, 529, 538  
 Fitzgerald Guy, 348  
 Flenghi Lucas, 427, 633, 762  
 Floridi Claudia, 574, 577  
 Folland Doug, 369, 371  
 Forbes Neil A., 674, 686, 692  
 Forsman Johan, 351  
 Foster J.D., 694  
 Fuchs-Baumgartinger A., 728  
 Fuller Rory G., 360  
 Fusellier Marion, 731

## G

Gaillot Hugues, 627, 753  
 Gaio Carlotta, 439, 603  
 Galhardo Franco Guilherme, 453  
 Galosi Livio, 476, 663, 689, 773, 774, 790  
 Galov A., 820  
 Galt Steffi, 619  
 Gambirasio Raffaella, 420  
 Gancz Ady Y., 691  
 Gandar F., 741

Garcia Párraga Daniel, 591  
 Garlet Maysa, 777  
 Gartner Anna Maria, 656  
 Gasthuys E., 357  
 Gatel Laure, 452, 469  
 Gaunt Stephen D., 360  
 Gehring R., 768  
 Gethöffer F., 523  
 Ghorbani Amir, 667  
 Gilabert J.A., 594, 740  
 Giner Jacobo, 443, 748  
 Giorgiadis Mariane, 461  
 Girod-Rüffer Christiane, 810  
 Giza L., 511  
 Globokar-Vrhovec Majda, 406  
 Goloni Camila, 448  
 Gombač Mitja, 356, 749, 750  
 Gomes Eymeric, 731  
 Gomes Sprada Arícia, 458  
 Gomez Guijarro Alicia, 359, 416, 442, 444  
 Gómez Ochoa Pablo, 782  
 Gonçalves Dias Luis Gustavo Gosuen, 453  
 González F., 775, 776  
 González González Enrique, 805  
 González González Fernando, 492, 779, 782, 781  
 González-Gasch Esteban, 755  
 Gottis A., 741  
 Gottstein Željko, 791  
 Grandis A., 607, 807  
 Grayson Doss, 696, 702  
 Greco Adelaide, 773  
 Groeneveld E. (Erik), 616  
 Grosset Claire, 739  
 Grosso Lisa, 774  
 Guillier D., 717  
 Guillon Leslie, 627, 745, 753  
 Gumpenberger M., 266, 556

## H

Haas Heiko, 440  
 Haberfield J., 403  
 Haddad Marandi Mohammadreza, 665, 667, 669  
 Haddock Sally, 369  
 Haesendonck R., 350, 511, 768  
 Hagedorn A., 709  
 Hahn Harriet, 417  
 Hammond J., 744  
 Hanzlíček Andrew, 364, 613  
 Harari Vivi, 691  
 Harcourt-Brown Frances, 641  
 Harcourt-Brown Nigel, 530, 678  
 Harcourt-Brown Tom, 641  
 Harries J., 426  
 Hartzler Kate, 708  
 Hasanzadeh Mohammad, 693  
 Haugwitz Patrick, 406  
 Hauptman Karel, 210  
 Hawkins Michelle G., 425  
 Head Keeper, 480  
 Heatley J. Jill, 507  
 Heckers Kim Oliver, 582, 583, 588

Heckmann Julia, 656  
 Hedley Joanna, 200, 752  
 Heffels-Redmann Ursula, 523, 656, 658  
 Heiden Michael, 440  
 Heimann M., 640, 735  
 Hellebuyck T., 511  
 Henriques Carlos, 407  
 Herden Christiane, 656, 658, 661  
 Hermans K., 738  
 Hernández Alberto, 479, 776  
 Hernando Elena, 766  
 Herzog Sibylle, 656, 658  
 Hetz S., 473  
 Hidalgo Antoine, 633, 762  
 Higbie Christine T., 363, 386  
 Hillemann Doris, 526  
 Hintze Barbara, 807  
 Hochleithner C., 266  
 Hoey Seamus, 621  
 Hoffmann Bernd, 380, 660  
 Hoitsy Márton, 806  
 Holland Larinda, 364  
 Hollandt Tina, 406  
 Horowitz Igal, 480  
 Horvatek Tomić Danijela, 791, 809  
 Houben R., 350  
 Houdellier Blandine, 452  
 Hsu Ed, 369  
 Huguet Baudin Eduardo, 591  
 Husson J.-C., 745  
 Huynh Minh, 417, 418, 674, 711, 731, 736

## I

Inurria Alicia, 794, 795  
 Ipsen J.D., 784  
 Iraola G., 390  
 Iwao Yamada Diego, 453  
 Izembart Anne, 584, 636

## J

Jäger Kathrin, 526  
 James Fiona, 690, 710  
 Jańczak Dawid, 811, 813  
 János Gál, 790, 806  
 Jayson Stephanie, 451  
 Jekl Vladimir, 166, 210  
 Jelić Dušan, 721  
 Jenckel M., 380  
 Jesus Sandra, 492  
 Johnson Laura, 496  
 Johnson R., 143  
 Johnson Stephen M., 708  
 Joyner LoraKim, 476  
 Juan-Sallés Carles, 479, 748  
 Jujan Angela, 494  
 Justice Will S.M., 464, 472

## K

Kabakchiev Claudia, 687, 690, 743  
 Kanda Ian, 364, 365, 517, 590, 613

Kaufman Elizabeth, 571  
 Kawanami Aline Eyko, 458  
 Keeble Emma, 619  
 Kelleher Susan, 369  
 Keller Dominique, 470  
 Keller Krista A., 425  
 Kempf H., 709  
 Kennedy R., 529  
 Keshavarz Samaneh, 693  
 Kharbush Rima, 708  
 Kik M.J.L. (Marja), 638  
 Kimyon Rebecca, 708  
 Kirmair R., 473  
 Kischinovsky Michelle, 675  
 Kitamura Kawamoto Fernando Yoiti, 453, 458, 466  
 Kliszcz Jakub, 813  
 Klopffleisch Robert, 807, 809  
 Knotek Zdenek, 94, 143, 542, 543, 544, 546, 558, 560, 572, 583, 588, 707, 723, 781  
 Knotková Zora, 583, 588  
 Koeppl Katja N., 682  
 Kolb Hélène, 432, 720  
 Kolesnik Ekaterina, 378, 402  
 Kölle P., 473  
 Konjević D., 820  
 Könyves László, 806  
 Korbel Rüdiger, 72, 373, 475, 485, 490, 660, 664, 670  
 Koroknai Viktoria, 563  
 Köstlinger Saskia, 614  
 Kramer Marc, 590  
 Krautwald-Junghanns M.-E., 473  
 Kromm Fred, 371  
 Kubiak Marie, 451, 464  
 KuKanich Butch, 606  
 Kummerfeld N., 473  
 Kunze A., 473  
 Künzel F., 728  
 Kuypers Marie, 419

## L

Laik-Schandelmaier C., 809  
 Landau Shmuel, 480  
 Langlois Isabelle, 348  
 Lanieste Delphine, 687, 690  
 LaRinda Holland, 613  
 Larrat Sylvain, 580, 637, 739  
 Latney La'Toya, 694  
 Le Bobinnet G., 733  
 Le Grange F., 617  
 Le Loc'h Guillaume, 666, 810  
 Le Souef A., 477  
 Ledwoń Aleksandra, 776  
 Lee Yen Tao, 713  
 Lefaux Brice, 461  
 Lennox Angela M., 369  
 Lepri E., 804  
 Levrier Céline, 427, 633, 762  
 Levy Alice, 637  
 Lichtenberger Marla, 53, 150, 307  
 Liebing J., 523  
 Lierz Michael, 509, 523, 538, 656, 658

Linsart Adeline, 418, 433, 634, 635  
 Linta N., 607, 807  
 Lock Brad, 575, 579  
 Loehr Victor J.T., 790  
 Lombardi Giovanni, 394  
 Lopes Filipa, 492  
 López Márquez Irene, 492, 781, 782  
 López-Yáñez María, 794  
 Lorenté F., 666  
 Loucachevsky Tatiana, 432, 584, 636, 720  
 Loureiro F., 366, 375  
 Lovati Silvia, 672  
 Lubian Emanuele, 120, 404, 574, 672  
 Lukač Maja, 721, 786, 791, 809  
 Lumeij J.T., 338  
 Lyngle-Cowand Kate, 371  
 Lyon Shane, 364

## M

Madani Seyed Ahmad, 665  
 Mader Douglas, 344, 714, 718  
 Magi Gian Enrico, 689  
 Magnone William, 766  
 Máinez Mireia, 479  
 Mäkitaipale Johanna, 623  
 Malberg Sara, 656, 658, 661  
 Manassero Matthieu, 419  
 Mancinelli Elisabetta, 213, 429, 441, 445  
 Manetti Cristiana, 577  
 Manfredi Maria Teresa, 404  
 Manon Steve, 810  
 Mans Christoph, 372, 441, 445, 496, 621, 702, 703  
 Mansoorlakooraj Hamed, 693  
 Marcianò Antonino, 650  
 Marcos S., 775  
 Marenzonia M.L., 792  
 Mari Subeide, 663, 780  
 Marlier Didier, 167, 741  
 Marquet Bertille, 457  
 Marrinhas Carla, 770  
 Marschang Rachel E., 378, 379, 402, 809, 810  
 Marshall Rob, 497  
 Martel A., 511  
 Martínez-Silvestre Albert, 387, 557  
 Martini M., 486  
 Martino Piera Anna, 603  
 Martorell Jaume, 557, 639, 729, 754, 766  
 Masi M., 381  
 Masson G., 529  
 Matas-Riera M., 569  
 Mathes Karina, 555  
 Matres-Lorenzo Luis, 634  
 Matt C., 395  
 Matteucci Giorgia, 768  
 Mawson P., 477  
 Mayer Jörg, 575, 589, 595  
 Mazza Giovanni, 420  
 McArthur Stuart, 642  
 McCain Stephanie, 525  
 McKinney Peter, 351  
 McManoman Rita, 525

McRee Anna, 360  
 Meinecke-Tillmann S., 538  
 Meinkoth James, 365  
 Melero Adrián, 387, 557  
 Melidone Raffaele, 418  
 Melillo Alessandro, 43  
 Mendonça Paula, 770  
 Mennonna Giuseppina, 773  
 Meomartino Leonardo, 773  
 Merchant Samer, 369, 371  
 MéridaMelvin, 476  
 Michelutti David J., 777  
 Miclard Julien, 584, 636  
 Mieritz Mark, 470  
 Millefanti Massimo, 574  
 Minh Huynh, 367, 368, 508  
 Miwa Yasutsugu, 632  
 Modesto Florent, 368, 418, 640, 717, 735  
 Moeremans I., 738  
 Moissonnier Pierre, 758  
 Moll J., 473  
 Monge Emma, 810  
 Montani Alessandro, 508  
 Montesdeoca Natalia, 780  
 Montesinos Andrés, 357, 359, 416, 444  
 Montesinos Barcelo Andres, 442  
 Morais M.E., 403  
 Morais Maria Eduarda Soares Lopes Fernandes, 722  
 Moraleda Fernández Virginia, 492, 779, 781, 782  
 Morandi F., 465  
 Moreno Miranda Isabel, 779  
 Morici Manuel, 543, 544, 572  
 Morphey T., 529  
 Morteza Haddadian, 669  
 Moser Kristina, 664  
 Mosteiro D., 366, 375  
 Müller Anja, 440  
 Müller Elisabeth, 810  
 Multon Julien, 758  
 Müller Kerstin, 807, 810, 816  
 Musgrave Kari, 517  
 Mutschmann Frank, 473, 809

## N

Nardini G., 552, 756  
 Nasiri Seyedmahdi, 693  
 Nava Eram, 665  
 Nebbia Patrizia, 394, 476, 528, 774  
 Nedeljković Gordana, 791  
 Nemetz Larry, 369  
 Neul Annkatrin, 378, 379  
 Nevarez Javier G., 114, 360, 363, 382, 386, 494, 710  
 Nicoletti Annalisa, 304  
 Nicolier Alexandra, 417, 418, 433, 565, 717, 733, 736, 762  
 Niero G., 390, 482, 486  
 Nili Hassan, 667  
 Nitzan Adam, 691  
 Nobrega Mario, 407  
 Nogradi Anna Linda, 563  
 Novellas Rosa, 557, 729  
 Noviello Emilio, 751, 773

Novikova E., 730  
 Nunes Telmo, 492  
 Núñez García Carmen, 779

## O

O'Brien Michelle F., 472  
 Oana Liviu, 704  
 Öfner S., 384  
 Olbert Marita, 661  
 Olive Mélanie, 720  
 Oliveri Matteo, 94, 542, 543, 544, 650, 707, 723, 572  
 Olivieri O., 725, 792  
 Origgi Francesco C., 790  
 Orós Jorge, 780, 794, 795  
 Orosz Susan E., 53, 84, 369, 476, 663, 689  
 Ortega Ojeda Andrés G., 580  
 Ott S., 709  
 Ouhmama R., 666

## P

Paepe D., 357  
 Pantchev Nikola, 406  
 Papa Caminiti Lucy Nicole, 441, 445  
 Paquet-Durand Isabelle, 476  
 Parisi Francesca, 420  
 Paterson S., 604  
 Paulsen Daniel, 382  
 Payton Mark, 364  
 Paz Avital, 571  
 Pearson Adam, 642  
 Pecchia Fabiana, 508  
 Pees M., 473  
 Pees Michael, 378, 379, 396, 582  
 Peighambari Seyed Mostafa, 665  
 Pelicella Fabio, 663  
 Pelizzone Igor, 412, 430  
 Pellikaan C.A. (Kees), 437  
 Pendl H., 38, 242  
 Penna Valentina, 802  
 Pérez-Merino P., 775  
 Pérez Orrico Marisa, 591  
 Perles Livia, 448, 453, 458, 466, 771  
 Perrin H., 426  
 PERRUCCI Stefania, 476, 773, 790  
 Peştean Cosmin, 704  
 Pesaro Stefano, 663  
 Peschel Andrea, 485, 664  
 Pesti Denise, 525  
 Pether Jim, 794  
 Petrini Daniele, 577, 759, 803, 804  
 Pfarrer Christiane, 555  
 Piccirillo A., 390, 482, 486  
 Pieper K., 473  
 Pignon Charly, 324, 418, 419, 637, 758  
 Pin Huan Yu, 713  
 Pinkerton Marie E., 496  
 Pirson S., 741  
 Pisa A., 775  
 Planellas Marta, 754  
 Plenz B., 473

Pompeu M.M.L., 788  
 PonceSantizo Gabriela, 476  
 Porcarelli G., 605  
 Porto Nigro Nicole, 777  
 Poser A., 486  
 Possenti Marzia, 420  
 Potier Romain, 432, 584, 636, 720  
 Prechsl Sebastian, 485  
 Prelaud P., 745  
 Preziuso S., 465, 780  
 Prieto M., 733  
 Proks Pavel, 546  
 Prukner-Radovčić Estella, 809  
 Pulici Stefania, 672  
 Pumarola Martí, 729  
 Putnam Mike, 369

## Q

Qi Soon X., 518  
 Quaranta Giuseppe, 512  
 Quarta P., 552, 756  
 Queiroz Pinho dos Santos Mariana, 466  
 Quintar Benoit, 461  
 Quinton Jean François, 627, 745, 753

## R

Rabsch Wolfgang, 396  
 Račnik Joško, 356, 749, 750  
 Radelof Katharina, 555  
 Rafferty A., 604  
 Rah Afrooz Anahita, 665  
 Raidal S., 477  
 Raiti Paul, 712  
 Raiwet Diana L., 348  
 Ramachandran Akhilesh, 395, 517  
 Ramirez Karen, 710  
 Rampacci E., 381  
 Rattiez Elise, 427, 633, 762  
 Ratti Gabriele, 672  
 Recio Alfredo, 729  
 Redaelli Elena, 603  
 Reese S., 473  
 Reginato A., 804  
 Regoli A., 349  
 Reichelt C., 473  
 Renfert Kevin, 396  
 Revuelta L., 775  
 Ribeiro Julia Maria, 448, 453, 458, 771  
 Ribeiro Sofia, 800  
 Ricci S., 465  
 Rickman Barry H., 734  
 Riddle K., 666  
 Riedel U., 473  
 Rinder Monika, 475, 485, 660, 664, 670  
 Risi Emmanuel, 432, 584, 636, 720  
 Rissi Daniel R., 589  
 Ritchie Branson W., 525  
 Rivera Sam, 579  
 Rizzi S., 547  
 Rizzi Theresa, 364

Robert Céline, 419  
 Robertson Jessica, 365, 517  
 Robic Mirna, 786  
 Robino Patrizia, 394, 476, 528, 774  
 Roccabianca Paola, 814  
 Rodenbaugh Cassandra, 364  
 Rodo Anna, 776  
 Rodrigues J.F.M., 410  
 Rodríguez Díez Clara, 781  
 Rodríguez Fernández Casilda, 781  
 Rodríguez J., 800, 804  
 Romain Sophie, 427, 633, 762  
 Römer-Oberdörfer Angela, 661  
 Romero Michael, 360  
 Roni Elias, 480  
 Ronot Pierre, 418  
 Ros-Rodríguez J.M., 594, 740  
 Rosenzweig Ariela, 480  
 Rossi Giacomo, 84, 476, 512, 528, 663, 689, 773, 774, 780, 790  
 Rossi Tiziana, 439, 603  
 Rossignoli Pedro Paulo, 458  
 Rosso Marilena, 820  
 Rota Simone, 398  
 Roy Alma, 386  
 Rozej-Bielicka Wioletta, 813  
 Rubbenstroth Dennis, 661  
 Ruel Y., 627  
 Ruel Yannick, 753  
 Ruelle Foucault, 637  
 Rui Patrício, 800  
 Ruiz Antonio, 794  
 Runge Jeffrey, 694  
 Runge Solveig, 661  
 Rush E. Marie, 580  
 Russo Riccardo Paolo, 711  
 Rusu Simona Mirela, 723

## S

Saam Nicole J., 485  
 Sabater González Mikel, 408, 591, 734, 755  
 Sabatini M., 349  
 Sałamatin Ruslan, 811  
 Salvitti de Sá Rocha Tiago André, 453  
 Sammarini Carolina, 766  
 Sanchez Susan, 595  
 Sanchez-Migallon Guzman David, 425  
 Sandmeier Peter, 361  
 Sandri Camillo, 766  
 Santoro Domenico, 751  
 Sargo R., 366, 375  
 Sargo T., 366  
 Sarpataki Orsolya, 704  
 Saunders D.A., 477  
 Saunders Jimmy, 452, 469, 640, 735  
 Saunders Richard, 451  
 Savarino Paolo, 814  
 Scaglione Frine, 512  
 Scarpona Silvia, 689  
 Schauburger Günther, 358  
 Schilliger Lionel, 143, 266, 565  
 Schlottau Kore, 380, 660  
 Schmidt Adam, 369  
 Schmidt L., 523  
 Schmidt Volker, 378, 379, 473  
 Schmidt-Ukaj S., 556  
 Schmitz Anna, 475, 664, 670  
 Schmitz Ornés A., 473  
 Schneider H., 509, 538  
 Schnitzer Petra, 277, 547  
 Schoemaker N.J. (Nico), 59, 192, 437, 616, 638  
 Schöniger S., 809  
 Schwarz Tobias, 619  
 Schwendenwein Ilse, 358, 728  
 Scope Alexandra, 358  
 Scott David E., 478  
 Scuota S., 792  
 Segond Sophie, 432, 720  
 Selleri Paolo, 508, 605, 623, 704  
 Semin Marie-Odile, 810  
 Sgariglia Elisa, 398  
 Sheen Joanne, 761  
 Shimizu Naomi, 674, 686, 755  
 Siebert U., 523  
 Sienkiewicz Beata, 776  
 Silva F., 366, 375  
 Silvanose C., 673  
 Silversides David W., 348  
 Silvestre Albert Martinez, 572  
 Silveti Sergio, 464  
 Sim Richard, 525  
 Simamura Ana Carolina Abon Ali, 466  
 Simonini C., 552, 756  
 Sirri R., 552, 756  
 Škorič Mišo, 588  
 Sladakovic Izidora, 90, 570, 595, 688  
 Sladky Kurt K., 202, 470, 496, 696, 708  
 Slana Iva, 781  
 Smit Elad, 480  
 Sobczyński Jacek, 813  
 Sobing A., 473  
 Sochorcova Veronika, 546, 560, 781  
 Sokolova Yuliya, 382  
 Sorrenti Loredana, 434  
 Sos Endre, 563  
 Soto Carlos, 394, 528  
 Sousa L., 366, 375  
 Spadola Filippo, 143, 543, 544, 572  
 Speer Brian L., 80, 295, 325, 369, 508  
 Spiezio Caterina, 766  
 Šprem N., 820  
 Staeheli Peter, 661  
 Stahl Scott J., 316  
 Stanclova Gabriela, 358, 728  
 Stanicki Kacper, 811  
 Starck J. Matthias, 379  
 Staudacher M., 809  
 Stec-Phillips Nicole, 590  
 Steen S., 604  
 Stefanetti V., 792  
 Steidler Stephanie, 820  
 Stepien Rebecca L., 621  
 Stidworthy Mark F., 400, 408, 464, 692

Stipoljev S., 820  
 Stöhr Anke C., 406  
 Stout Rhett, 710  
 Straub Jens, 526  
 Strobel S., 709  
 Strokowska Natalia, 617, 813  
 Suárez Regalado Laura, 492, 781, 782  
 Summa Noémie M., 425  
 Švara Tanja, 356, 749, 750  
 Sypniewski Lara, 590, 613  
 Sys S., 350  
 Szabo Zoltan, 216  
 Szeleszczuk Piotr, 776  
 Szikszai P., 728  
 Szulc Jacek, 813

## T

Tagliavia C., 607, 807  
 Takimoto Haruka, 632  
 Tecilla Marco, 429, 814  
 Tena Pérez Gloria, 779  
 Teso Sanchez Beatriz, 359, 416, 442, 444  
 Testault Isabelle, 584  
 Thas Inge, 424  
 Thas Olivier, 424  
 Thierfelde Stephan, 475  
 Thilliez Natacha, 739  
 Tischbirek D., 473  
 Tomás J., 366, 375  
 Tosta María Carolina, 795  
 Tozon Nataša, 356, 750  
 Tramuta Clara, 394, 528, 774  
 Tully Jr. Thomas N., 360, 363, 494  
 Tünde Pilis, 790  
 Turk Romana, 786

## V

Vaidlova H., 783  
 Valentini Andrea, 398  
 Valdeperes M., 776  
 Vallefucio Rosario, 324  
 van den Berk S.G.M. (Sabine), 638  
 van der Sluis M. (Malou), 515  
 Van Soom A., 511  
 van Steyvoort Olivia, 367  
 Van Wettene A.J., 784  
 van Zeeland Y.R.A. (Yvonne), 59, 192, 437, 515, 616, 638  
 Velarde Roser, 387  
 Vella David, 761  
 Verdaguer I., 387  
 Ververs C., 511  
 Vetere Alessandro, 574  
 Vignoli M., 552, 756  
 Vilhena Hugo, 770  
 Villa Demetrio Lais, 777  
 Vinherkar Dinesh, 585, 796  
 Vitali S., 477

Vitolo Gaetano Daniele, 802, 815  
 Vobornik Angela, 358  
 Völker I., 523  
 von Degerfeld Mitzy Mature, 512  
 Vossen J.P.H.M. (Jos), 437  
 Vranković L., 820

## W

Wagner Robert, 424  
 Wall C.R., 395  
 Wallace Michiko, 371  
 Waller Kenneth R., 621  
 Warchulska Karolina, 813  
 Warren K.S., 477  
 Washburn Wesley, 369  
 Watanabe Minto Bruno, 453, 458, 466  
 Watters Jyoti, 708  
 Waugh D., 509  
 Webster M., 529  
 Wehrend A., 538  
 Weiffenbach G., 809  
 Weijnenborg Campos Lilian, 458  
 Weiß J., 816  
 Wellehan Jr. James F.X., 382  
 Wencil P., 242  
 Werther Karin, 448, 453, 458, 466, 771  
 Whitehead M.L., 529  
 Wilcox Crystal, 371  
 Wilhelm A., 784  
 Williams Jackie M., 372  
 Winkel-Blair Andrea, 507  
 Wissink-Argilaga N., 569  
 Witt Sandra, 614  
 Wohlsein P., 523  
 Wolfensohn Sarah, 472  
 Wolfesberger B., 728  
 Wolff Bernard, 525  
 Wright Loudon, 606  
 Wu Ruey-Shyuan, 630  
 Wuest E., 658  
 Wuest E., 67

## Y

Yun Shan Chiu, 713

## Z

Zadravec Marko, 356, 749, 750  
 Zanzani Sergio, 404  
 Zap Adrien M., 580  
 Željko Gottstein, 809  
 Zemanova Anna, 558, 560  
 Zicavo A., 792  
 Ziegler L., 523  
 Zoller Graham, 367, 368, 417, 674, 731, 736  
 Zoltán Orosi, 790, 806  
 Zur Linden Alex, 687, 690



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